

**THE PHARMACOLOGY AND PHYSIOLOGY OF NITRITE AND NITRATES**

**By**

**Maurice M. Rath**  
M.D.

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THE RELATIONSHIP BETWEEN AGE AND BLOOD NITRITE CONCENTRATION IN HUMAN SUBJECTS (202)

THE PERCENTAGE FALL IN BLOOD PRESSURE AND RATE OF DISAPPEARANCE OF NITRITE AFTER INTRAVENOUS ADMINISTRATION IN DOGS

THE EFFECT OF ORAL AND INTRAPERITONEAL ADMINISTRATION OF SODIUM NITRITE UPON THE BLOOD PRESSURE IN UNANESTHETIZED HYPERTENSIVE RATS



## INTRODUCTION

Most standard pharmacology text books (Bastedo 1938, Cushny 1940, McGuigan 1940, Solis-Cohen and Githens 1938, Sollmann 1937) teach that nitrites effect a fall in blood pressure by lessening the tone of arterial muscle, which in turn causes peripheral vasodilatation. Also, it is generally stated that the nitrates are converted into nitrite before they act as "hypotensive" agents in the body. One recent text on the general subject by Goodman and Gilman (1941), however, does state that nitrites act on the arterioles, capillaries and venules, but that the effect is more marked on the postarteriolar vascular bed.

Recent years have witnessed a renewed interest in the pharmacology of nitrites. Work has been reported which places in controversy several of the heretofore-accepted views about this subject. Krantz, Carr and coworkers (1939, 1940) have presented data which tend to show that the action of erythritol tetranitrate, glyceryl trinitrate and mannitol hexanitrate in reducing the blood pressure of dogs is not dependent upon hydrolysis and nitrite formation, but rather on the unhydrolyzed molecule.

Others (Wilkins et al. 1937, Stead et al. 1939, Chasis et al. 1937, Smith et al. 1938) contend that the action of nitrites is chiefly on the venous rather than on the arterial system. Weiss and Wilkins (1937) believe that sodium nitrite may cause arteriolar constriction and that the fall in blood pressure produced by this drug is due to the reduction in venous tone, and as a consequence, to the pooling of blood in the peripheral veins. The changes produced by nitrite involve, they state, the vascular bed peripheral to the arterioles, i.e., capillaries, venules and veins (Wilkins et al. 1937). The arteriolar constriction results secondary to the reduction of arterial pressure, through the carotid

sinus mechanism. Earlier research (Burton-Opitz and Wolf 1910, Capps and Matthews 1913) also showed that nitrites produce a fall in venous pressure which is attributable to direct influence on the venomotor mechanism. It has been reported that even when circulatory collapse occurs from susceptibility to nitrites or from overdosage, postarteriolar rather than arteriolar dilatation is the cause. The arterioles may be constricted due to compensatory vasomotor reflexes (Wilkins et al. 1938).

Another interesting point about the action of nitrites has been reported by Chasis, et al. (1938) who found no evidence of renal participation in the vasomotor responses induced by sodium nitrite. He and his coworkers found that after administration of 15 milligrams of sodium nitrite per kilogram per minute intravenously, the blood pressure was reduced, but no circulatory disturbances occurred when the subject was in the recumbent position. When the subject was raised to 45 degrees, the blood pressure dropped to a level of 66 millimeters systolic and 44 millimeters diastolic, and signs of circulatory inadequacy appeared.

Finally, that nitrites are capable of reducing the blood pressure in therapeutic or safe doses has been contested. Grollman, Harrison and Williams (1940) have stated that sodium nitrite and erythrol tetranitrate do not lower the blood pressure in unanesthetized hypertensive rats, and that the clinical value of these and related substances is thus questionable. They maintain that such drugs advocated in the past as effective agents in reducing the blood pressure probably induce a significant depression of blood pressure only when used in toxic doses. The view that these substances effectively lower blood pressure is

based, according to these authors, upon early experiments performed on man or upon the usual effects observed in experimental anesthetized animals. They point out in the case of the anesthetized animal the blood pressure decline is probably conditioned by a failure of the heart to respond due to a disordered state of the circulation induced by the anesthetic. These investigators administered sodium nitrite and erythrol tetranitrate in doses of 0.1 gram and 30 milligrams, respectively, per rat per day, mixed with the animal's food. It is well recognized that the action of nitrites is comparatively short-lasting and it is quite possible that any detectable effects of the drugs may have worn off during the time consumed in preparing the animal prior to taking its blood pressure. Besides, with the doses employed only very small amounts of the drug were ingested at any given time, perhaps too little to effect any appreciable reduction in blood pressure.

In the normal human subject, also, investigations have attempted to show that amyl nitrite (Lindhard 1915) or sodium nitrite (Gaisbock and Jarisch 1927) do not appreciably lower the blood pressure when administered in therapeutic doses. In this connection, Rzentkowski (1909) demonstrated that flushing of the skin of the head and neck may occur after administration of nitrite in doses which cause no fall in blood pressure. He suggested that the vessels in this area of the skin are most susceptible to these drugs. Others contend that amyl nitrite acts primarily upon the cerebral vessels--more so than the other nitrites (Norris, Bazett, and McMillan 1927).

Most of the literature supports the view that nitrite acts, in vitro, to relax smooth muscle without reference to innervation. However, Macht (1914) and Love, McGuigan, and Wiley (1925) found that nitrite caused contraction of the excised pulmonary artery.

A search into the literature has revealed that very little, if anything, is known concerning the normal physiological significance of the nitrite ion, i.e. whether or not the nitrite ion exists as such in the circulation and if it does, what role it plays and what factors influence its concentration in the blood.

Studies by several investigators indicate that the nitrite ion does exist normally in blood. Stieglitz and Palmer (1934, 1937) determined its presence in the blood of normal human subjects but obtained consistently negative results when dog and rabbit blood was analyzed. These observers frequently noted the absence of nitrite in human blood during the warm months of the year. They reported an average concentration of 1:100,000,000 for 26 subjects during the cool months.

An error in the mathematical treatment of their data was discovered and reported to the authors by this department. Stieglitz has agreed that their values require correction (1941). By their method of preparing a sodium nitrite standard for comparison, Stieglitz and Palmer (1934) made a 1:100 dilution of a stock solution containing 0.02 gram of sodium nitrite per liter. They stated that the final dilution contained 0.00114 milligram nitrite per 100 cc. whereas the latter figure should have been 0.01, a ten-fold error. Furthermore, in their calculation of the quantity of blood nitrite from colorimetric readings a dilution factor was neglected. Since a dilution of 1:4 was made of blood by their method during the process of precipitating the proteins, a dilution factor of 4 should have been employed, (instead of the figure 0.5, which they used).

Extensive analyses for nitrite in normal blood have not, therefore, been heretofore reported. Before this and other phases of the problem could be studied, however, an efficient method for the quantitative deter-

mination of nitrite in blood had to be devised.

In summary, the more recent pharmacological research has raised the following queries: How can the nitrite level in blood be readily determined? To what extent does nitrite exist in blood and what factors influence its concentration? What is the physiological role of the nitrite circulating normally in blood? Are the nitrates which are employed for reducing blood pressure converted in the body to nitrite before they are capable of performing this function? After administration of nitrite, is the blood pressure dependent upon the level of nitrite in the circulating blood, i.e., is there a correlation between the blood nitrite concentration and blood pressure? Are nitrites effective at all in lowering the blood pressure in unanesthetized animals? The purpose of this investigation is to shed light upon these and other questions which up to the present remain unanswered.

## METHOD FOR QUANTITATIVE ANALYSIS OF BLOOD NITRITE

The method, in order to be successfully applicable for this study, should comply with the following requirements: (1) it has to be sufficiently sensitive for detecting extremely minute amounts of nitrite, (2) the method itself must not destroy nitrite and (3) it should be capable of determining nitrite in blood.

Stieglitz and Palmer (1934) developed a colorimetric test for blood nitrite, employing *a*-naphthylamine and the disodium salt of *b*-naphthylamine-6, 8-disulfonic acid ("amino G" acid), which they claimed to be more sensitive and more accurate than the Ilosvay reaction (1889). The method of Stieglitz and Palmer, as studied in this laboratory, was found no more sensitive than the Ilosvay reaction for nitrite. According to these investigators, the minimum concentration which yields a positive color with the Ilosvay reaction is 0.0001 gram of nitrite per liter, or one part in 10 million. Using their own method they state that a detectable color is noted with 0.000005 gram per liter, or one part in 250 million. Richardson and Hollings (1903), using sulfanilic acid and *a*-naphthylamine, were able to estimate the color produced by as little as 1 cc. of a solution containing 1 part of nitrite per 10 million. They employed a Lovibond tintometer for comparison. Treadwell (1935) and Snell (1936), in addition, states the Ilosvay method to be sensitive up to one part per billion. This finding has been confirmed in this laboratory. By a slight modification of the Ilosvay procedure (to be described presently) a higher sensitivity has been attained.

Stieglitz and Palmer claim that "The color of the Ilosvay reaction fades so rapidly that a fresh standard must be prepared for each

determination \_ \_ \_". In the first place, no fading was noted in this laboratory until after hours of standing. Snell (1936) states that the color produced by the Illosvay method is reliable for 30 to 45 minutes. Secondly, a fresh standard or standards should be prepared for each determination, and in exactly the same manner as the sample to insure accurate results.

Criticism of the mathematical treatment which Stieglitz and Palmer gave to their data has been previously made. Stieglitz (1941) has agreed that an error does exist. Several criticisms of their own method, as described in their paper (1934), follow:

- (1) The standard color for comparison was not prepared in the same manner as the blood filtrate; i.e., the protein precipitating reagents, zinc sulfate and sodium hydroxide, were added to the sample of blood but not to the standard solution. These chemicals impart a color shade different from that produced by a reaction in their absence, making matching difficult as well as inaccurate in the ordinary colorimeter.
- (2) These authors used one standard nitrite solution for the purpose of comparison. In view of the typical colorimetric readings as reported in their paper, standards of more suitable concentration should have been used for more accurate results, i.e., the levels of the two plungers of the colorimeter were too far apart.
- (3) Their employment of silver nitrite in the preparation

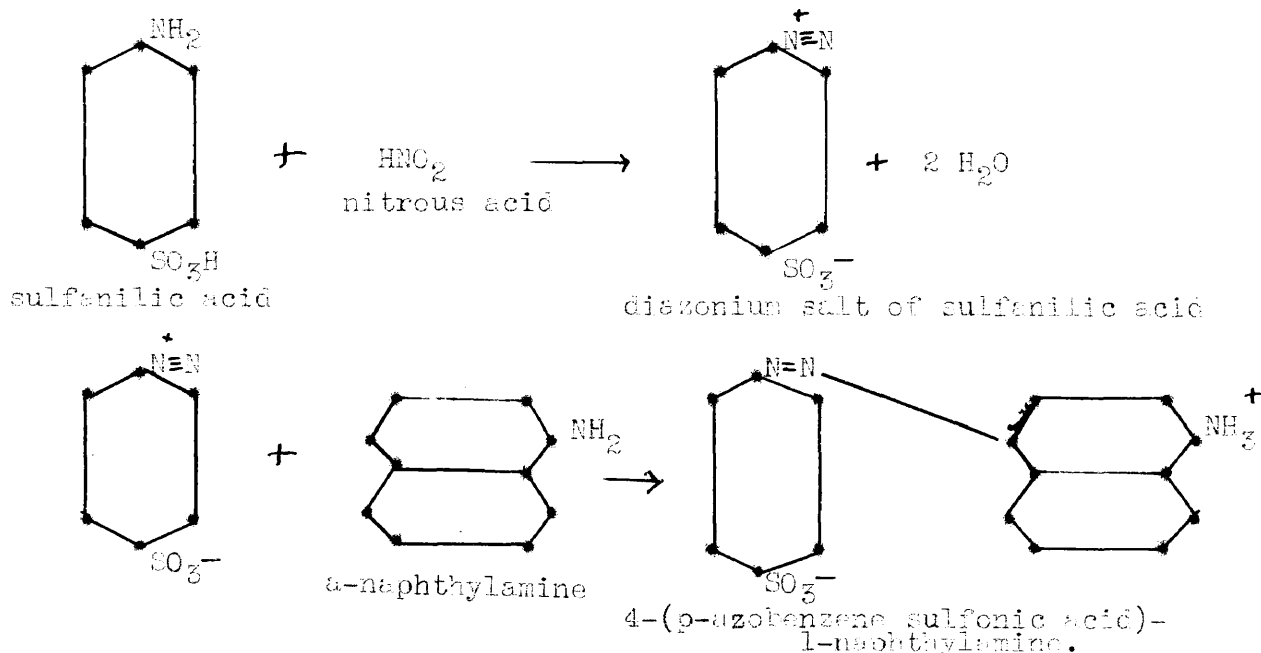
of nitrite standard solutions is open to criticism. In the light of recent evidence, (Reindollar 1940) its use is likely to impair the accuracy of results. This point will be further discussed presently.

- (4) The  $\alpha$ -naphthylamine solution in the high concentration (0.1 gram in 20 cc. of distilled water) suggested by Stieglitz and Palmer is difficultly prepared, is extremely unstable, and turns pink and clouded within a short time.
- (5) The "amino G" acid solution is made with too indefinite a final concentration, the authors stating that " \_ \_ about one small drop of the commercial 32.6 per cent solution of the disodium salt of amino-G acid is added to 50 cc. of pure distilled water, yielding a pale opalescent solution." Under such conditions it is conceivable that this reagent may yield inconsistent results.

A modification of the Ilosvay reaction proved sufficiently sensitive for our purposes and capable of being more efficiently executed. Ten cubic centimeters of a nitrite solution containing 0.1 gamma per 100 cc. (1 part per billion) gives, by this method, a definitely detectable color reaction. Treadwell (1935), in contradistinction to the report of Stieglitz and Palmer (1934), found the Ilosvay test sensitive to one part nitrite per billion, using 50 cc. of a nitrite solution containing 0.001 milligram per liter.



Principles of the Method - Sulfanilic acid and  $\alpha$ -naphthylamine are employed, which in the presence of nitrous acid, react to produce a violet azo dye. In the process the sulfanilic acid is converted by the nitrous acid into the corresponding diazo compound and the latter reacts with the  $\alpha$ -naphthylamine to form  $\alpha$ -naphthylamine-*p*-azobenzene-*p*-sulfonic acid or 4-(*p*-azobenzene sulfonic acid)-1-naphthylamine.



The details of the method are described under five headings: (A) Preparation of Protein-free Blood Filtrate, (B) Production of Color Reaction, (C) Preparation of Standards for Comparison, (D) Colorimetric Reading, and (E) Precautions.

(A) Preparation of Protein-free Blood Filtrate\*- Since the nitrite ion is unstable in strongly acid media, the method of removing the blood proteins must not be associated with a low pH. Somogyi (1930) devised a satisfactory means for accomplishing this end using zinc sulfate and sodium hydroxide. The high dilution, however, of blood resulting after addition of the reagents in the concentrations prescribed by him is un-

\*Supernatant fluid is to be understood wherever this term is used.

desirable since the nitrite ion is present in extremely minute amounts in normal blood. The modified method of Stieglitz and Palmer (1934) was employed.

By this method, one volume of blood is thoroughly mixed with two and a half volumes of 4.5 per cent zinc sulfate solution in an Erlenmeyer flask. One-half volume of 1 N sodium hydroxide is added and the mixture again shaken. Centrifugation for approximately fifteen minutes at a speed of 3,000 r.p.m. produces a clear filtrate. If immediately after blood is withdrawn from a blood vessel it is placed in the flask containing the zinc sulfate, no special anticoagulant need be employed since the zinc sulfate serves this purpose.

For most of the analyses on normal blood, 10 cc. samples were studied which yielded 20 cc. of filtrate (equivalent to 5 cc. whole blood). In those experiments where nitrite was injected, only 3 or 4 cc. of blood was employed and 1 or 2 cc. of the filtrate proved sufficient for analysis.

(B) Production of Color Reaction - A definite quantity of the clear, protein-free filtrate is placed in a tall 50 cc. Nessler tube containing 2 cc. of each diazotization reagent,  $\alpha$ -naphthylamine<sup>1</sup> and sulfanilic acid<sup>2</sup>. The Nessler tube is then placed in a beaker of water maintained at 75-85° C. and allowed to remain for 10 minutes. The tubes containing the standards for comparison are prepared in exactly the same manner, i.e., a quantity of filtrate exactly the same as that obtained from blood is employed, such filtrate being obtained by mixing an equal amount of

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<sup>1</sup> $\alpha$ -naphthylamine solution is prepared by placing 100 milligrams of the base (Eastman Kodak Co., N.Y.) in boiling 5 N acetic acid. Store in tightly stoppered glass container.

<sup>2</sup>Sulfanilic acid solution is prepared by dissolving 0.5 gram in 150 cc. of boiling 5 N acetic acid.

standard solution, in place of blood, with zinc sulfate and sodium hydroxide.

(C) Preparations of Standards for Comparison - Nitrite standards in the past have been prepared from silver nitrite by interaction with sodium chloride (Treadwell 1935, Mason and Euswell 1931, American Public Health Association 1936). The reason given for this procedure was based on the questionable purity and deliquescent nature of the then-available sodium nitrite. Reindollar (1940), however, has recently demonstrated that sodium nitrite of high quality may be readily secured and may serve as the primary standard in nitrite analysis. Furthermore, he points out, the use of silver nitrite in the preparation of nitrite standards entails definite difficulties which are likely to impair the accuracy. This salt is somewhat photosensitive, deteriorates slightly with age, and may become unreliable as a standard. It dissolves slowly and with difficulty. Sodium nitrite, on the other hand, is relatively inexpensive and solutions of it can be quickly prepared fresh as often as required. In this manner the problem of deterioration arising from the use of an old nitrite solution is obviated.

Merck's reagent grade granular sodium nitrite (99.4 per cent) was employed in this laboratory. After making due allowance for the slight amount of impurity, a stock solution was prepared containing 1.0 milligram of the nitrite ion per 1.509 cc. of solution by dissolving 1.0 gram of sodium nitrite (99.4 per cent) in 1000 cc. of nitrite-free water. From this stock solution successive dilutions were made to obtain the necessary standards for comparison. No deterioration of the stock solution could be detected after standing two weeks, but in order

to insure constant results it was prepared fresh each week and the various dilutions serving as standards were made daily. When analyzing for nitrite in normal blood (animals or subjects receiving no nitrite administration), 8 standards were used, ranging in concentration from 0.5 to 15 gamma of nitrite per 100 cc.

(D) Colorimetric Reading - When analyzing small quantities (5-10 cc.) of solutions containing nitrite concentrations of less than 1 part per 10,000,000, colors are produced which are too faint for matching in the ordinary colorimeter. Tall 50 cc. Nessler tubes and a Nesslerimeter<sup>1</sup> were used for this purpose and provided a method which is rapid, convenient and accurate. Chances of contamination by this procedure are minimized because the solution containing the nitrite ion can be mixed with the azo dye reagents in one Nessler tube, the mixture then heated in a water bath, and the color so developed compared with standards solutions --- without transferring to any other container. As many standards of varying concentrations can be employed as are necessary and placed in the Nesslerimeter for matching colors.

Colorimetric matching, by means of the Nesslerimeter, was made on samples of normal blood. When the concentration of nitrite is more than 15 gamma per 100 cc. the ordinary colorimeter with 50 millimeter

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<sup>1</sup>The Nesslerimeter (Fisher Scientific Company, Pittsburgh, Pa.) consists of a base with a housing containing a daylight blue lamp for illuminating with equal intensity both samples being compared. A support has holders for ten Nessler tubes which can be employed for containing standard solutions of graduating concentrations. Each tube is shielded from extraneous light. Light beams enter the bottom of the Nessler tubes, pass through both the liquid being examined and the standard, then emerge and are observed in juxtaposition in the eyepiece of the instrument after encountering a mirror and lens system. The Nessler tubes are uniform and have glass bottoms of equal thickness. The columns of liquid in the tubes were usually 13 cms. high. The support of the Nesslerimeter can be rotated for obtaining the color match.

cups is suitable. In the latter case, fewer standards of varying concentration need be employed.

By the method herein described, readings were reproducible when analyses were made on 10 samples of blood withdrawn from a single pool of blood.

(E) Precautions - Great care must be exercised against contamination with nitrites from various sources. The nitrite ion exists in the air of any laboratory where concentrated nitric acid is employed, and uncovered glassware may be readily contaminated. In fact, exhalation of the smoke after puffing a cigarette liberates sufficient nitrite to produce a color reaction when collected in a test tube containing the diazotizing reagents. Therefore, all apparatus must be cleaned with cleaning solution followed by careful rinsings with distilled (nitrite-free) water immediately prior to use. Fresh-distilled water, tested for freedom of nitrite, was used for the preparation of reagents and solutions, as well as for rinsing. Tap water contains a relatively high nitrite concentration and is hence definitely unsuitable for this work. Blanks should be simultaneously tested for contamination.

Nitrite-free water can be prepared by distilling water over a mixture of potassium permanganate and potassium hydroxide (50 cc. of a mixture containing 5 per cent potassium permanganate and 35 per cent potassium hydroxide diluted up to a liter with distilled water; the distillate collected in the usual manner). Fresh-distilled water, as stated previously, is suitable.

## DETERMINATION OF NITRITE IN THE BLOOD OF THE DOG, STEER, MONKEY, AND MAN

By the method described in the foregoing pages for determining minute quantities of nitrite, the blood of various species of animals was analyzed. TABLES I, II, III and IV present the results of this investigation for the dog, cow, rhesus monkey, and man, respectively.

For 69 dogs, the average nitrite level in the blood obtained either from the external jugular vein or femoral artery was 9.5 gamma, plus or minus a standard error of 0.39, per 100 cc. of blood.

The blood of the steer, collected from the jugular vein at the time of slaughter, contained an average 9.4 gamma of nitrite per 100 cc. for 5 steer with a standard error of 1.25 gamma.

Fourteen monkeys from which samples of blood were obtained from the ventricles of the heart had a nitrite concentration averaging 11 gamma per 100 cc. plus or minus a standard error of 0.5.

A study of a series of 170 human subjects revealed an average of 9.45 gamma of nitrite plus or minus a standard error of 0.47 per 100 cc. of blood withdrawn from the antecubital vein. These individuals were patients hospitalized for traumatic injuries, operative treatment etc. Both sexes, white and colored races, all age levels and various blood-pressure levels were represented in this series of individuals. The records of these same subjects will serve as a basis for further statistical analysis in the following section of the thesis.

On the basis of these results it appears that nitrite is present normally in the blood of the dog, steer, monkey, and man, and it exists in approximately the same detectable concentration in all these species, namely 10 gamma per 100 cc. of blood, or one part in ten million.

Supporting the findings of Krantz, Carr and coworkers (1938), it was found that the blood of dog contains nitrite. These investigators did not determine the exact quantity of nitrite in blood in various species. They were interested in learning the relative increase in nitrite concentration after drug administration to dogs. Stieglitz and Palmer (1937) were unable to reveal the presence of nitrite in the blood of the dog. Also, these investigators reported that human blood frequently contains no nitrite in warm weather. It is to be noted that 90 per cent of the series of human subjects presented in TABLE IV was obtained during the summer months in Baltimore, Maryland.

Whether this detectable quantity of blood nitrite represents all of nitrite present in blood is as yet unknown. Stieglitz and Palmer (1937) have reported that protein decreases the amount of nitrite which can be measured, and that the greater amount of protein present, the greater decrease in measurable nitrite.

TABLE I

## BLOOD NITRITE CONCENTRATION IN DOGS

Dog Number	Nitrite in Gamma per 100 cc. Blood	Dog Number	Nitrite in Gamma per 100 cc. Blood
11	4	34	10
22	4	35	10
61	4	38	10
15	5	40	10
23	5	45	10
24	5	54	10
29	5	56	10
30	5	63	10
36	5	64	10
62	5	65	10
66	5	69	10
57	6	47	11
58	6	49	11
28	7	50	11
55	7	52	11
59	7	1	12
60	7	4	12
5	8	7	12
13	8	8	12
17	8	9	12
20	8	19	12
25	8	21	12
26	8	33	12
31	8	42	12
39	8	43	12
41	8	44	12
16	9	46	12
67	9	48	12
2	10	68	14
3	10	10	15
6	10	37	15
14	10	51	15
18	10	53	15
32	10	12	18

Total Number of Dogs	69
Mean	9.5
Standard Deviation	3.2
Standard Error	0.39



TABLE II

## BLOOD NITRITE CONCENTRATION IN THE STEER

Steer Number	Nitrite in Gamma per 100 cc. Whole Blood
1	13
2	10
3	10
4	7
5	7

Mean	9.4
Standard Deviation	2.5
Standard Error	1.25

BLOOD NITRITE CONCENTRATION IN RHESUS MONKEYS

TABLE III

Nitrite in Gamma  
per 100 cc. Whole Blood

Monkey Number

1	9
2	10
3	12
4	12
5	12
6	12
7	12
8	12
9	12
10	12
11	10
12	10
13	10
14	8

Mean  
Standard Deviation  
Standard Error

11

1.8

0.5

TABLE IV

## BLOOD NITRITE CONCENTRATION IN MAN

Sex-Color	Age	Nitrite in Gamma per 100 cc. Blood	Blood Pressure	Pulse Pressure
MC	76	0.5	150/94	56
MW	81	0.5	150/90	60
FW	91	0.5	204/100	104
MW	55	0.5	190/105	85
MW	41	0.5	180/105	75
MW	60	0.5	180/105	75
MW	53	0.5	150/100	50
FW	43	0.5	230/120	110
MC	64	0.5	190/140	50
FW	54	0.5	160/90	70
FW	60	0.6	210/110	100
FW	65	0.6	210/110	100
MW	72	0.7	200/100	100
FW	75	1.0	164/96	68
MW	72	1.0	160/90	70
MW	76	1.0	150/90	60
MW	71	1.0	150/80	70
FW	72	1.0	230/110	120
MC	50	1.0	205/110	95
MW	55	1.0	200/100	100
FC	66	1.0	190/110	80
FW	78	1.0	174/105	69
FW	56	1.0	222/148	74
MW	80	1.0	115/175	40
MC	55	1.0	116/70	46
MW	51	1.0	96/60	36
FW	40	1.0	140/80	60
MW	57	1.0	120/80	40
FC	28	1.0	125/80	45
MW	71	1.5	170/90	80
MW	67	1.5	180/90	90
FW	68	1.5	200/100	100
FC	65	2.0	230/110	120
MC	39	2.0	140/120	20
MC	54	2.0	160/100	60
FC	21	2.0	130/80	50
FW	41	2.5	130/60	70
FW	9	3.0	140/70	70
MW	37	3.0	120/60	60
MW	29	3.0	120/70	50
FW	16	3.0	120/78	42
MC	30	3.0	130/70	60

TABLE IV (Continued)

Sex-Color	Age	Nitrite in Gamma per 100 cc. Blood	Blood Pressure	Pulse Pressure
FW	21	3.0	112/80	52
MM	49	3.0	115/70	45
FW	40	3.0	140/80	60
FC	29	3.0	130/90	40
MM	63	3.0	120/80	40
MM	37	3.0	120/80	40
MM	72	3.0	200/85	115
FW	63	3.0	165/80	85
FW	46	3.0	160/80	80
FW	61	3.0	150/84	66
FW	24	3.0	190/120	70
FW	43	3.0	150/100	50
MM	43	3.0	170/110	50
MM	57	3.0	155/100	60
FC	54	5.0	180/120	55
MM	71	5.0	180/120	60
MM	70	5.0	190/120	70
MM	25	5.0	120/90	20
MM	25	5.0	150/70	60
MM	58	5.0	118/88	30
MM	26	5.0	120/90	30
FC	26	5.0	150/80	50
FW	42	5.0	124/76	48
FC	33	6.0	145/100	45
MC	41	6.0	125/95	30
FC	52	6.0	150/59	70
MC	52	7.0	190/90	100
MC	54	7.0	145/90	55
FW	35	7.0	145/95	50
MM	67	7.0	185/100	85
MM	50	7.0	175/135	40
PC	51	7.0	140/100	40
FC	20	7.0	120/70	50
FC	39	7.0	120/70	50
FC	38	7.0	120/70	50
FC	74	7.0	130/65	65
MM	74	7.0	130/70	60
MM	51	7.0	120/80	40
FW	11	7.0	130/84	46
MC	66	7.5	160/100	60
MC	76	8.0	140/90	50
MC	58	8.0	135/75	60
FC	64	9.0	110/60	50
FC	71	9.0	165/100	65
FC	48	9.0	135/95	40
FC	32	9.0	150/80	70
FC	62	10.0		

TABLE IV (Continued)

Sex-Color	Age	Nitrite in Gamma per 100 cc. Blood	Blood Pressure	Pulse Pressure
MC	90	10.0	152/84	68
FC	56	10.0	210/110	100
FC	65	10.0	190/100	90
MC	62	10.0	140/100	40
FC	50	10.0	165/105	60
MW	76	10.0	200/120	80
FW	13	10.0	126/78	48
FW	22	10.0	114/90	24
MW	20	10.0	120/80	40
MW	31	11.0	120/80	40
MW	54	11.0	140/80	60
MW	27	11.0	160/90	70
FC	57	11.0	150/90	60
FW	92	11.0	150/90	60
MC	65	11.0	145/95	50
MC	70	11.0	228/110	118
FC	54	11.0	155/90	65
FC	63	11.0	160/100	60
FW	62	11.0	145/100	45
FW	71	12.0	155/95	60
FW	31	12.0	130/98	32
FC	55	12.0	150/105	45
FW	18	12.0	80/20	60
FW	59	12.0	130/90	50
MW	36	12.0	120/80	40
MW	28	12.0	110/70	40
FW	12	12.0	120/75	45
MW	40	12.5	125/85	40
FC	49	13.0	120/70	50
FC	27	13.0	120/80	40
MW	23	13.0	134/84	50
FW	58	13.0	140/90	50
FC	59	13.0	140/90	50
FC	54	13.0	180/80	100
FC	60	13.0	170/90	80
FC	64	13.0	170/115	55
MC	66	13.0	150/110	40
FW	39	13.0	180/120	60
FC	69	14.0	215/95	120
MC	71	14.0	150/88	62
MW	52	14.0	150/110	40
MC	68	14.0	140/100	40
FW	38	14.0	118/58	60
MW	40	14.0	90/50	40
FW	40	14.0	130/70	60

TABLE IV (Continued)

Sex-Color	Age	Nitrite in Gamma per 100 cc. Blood	Blood Pressure	Pulse Pressure
MM	32	14.0	128/76	52
MM	25	14.0	128/76	52
MM	30	14.0	140/90	50
MM	18	14.0	120/80	40
MM	25	14.0	110/80	30
FW	25	15.0	122/60	62
FW	69	15.0	80/60	20
MM	58	15.0	140/84	56
MC	26	15.0	215/180	35
MM	20	15.0	146/46	100
FW	53	16.0	140/100	40
MM	40	16.0	155/106	50
MM	35	16.0	130/100	30
MM	50	16.0	120/70	50
MM	36	16.0	130/80	50
MM	20	16.0	130/80	50
MM	63	16.0	122/80	42
MM	44	16.0	130/84	46
MM	18	17.0	140/90	50
MM	25	17.0	114/60	54
FW	20	17.0	105/65	40
FW	23	17.0	128/74	54
MM	58	17.0	118/88	30
MC	64	17.0	180/110	70
MM	57	17.0	200/110	90
MM	30	17.0	145/60	85
FW	58	18.0	132/78	54
MM	26	18.0	110/60	50
FW	21	18.0	110/75	25
MM	32	18.0	130/90	40
FW	30	18.0	122/80	42
FW	33	19.0	116/66	50
FW	37	20.0	124/78	46
MC	25	20.0	160/120	40
MM	43	22.0	130/80	50
MM	52	22.0	125/75	50
MM	64	24.0	138/82	56
MM	42	24.0	240/180	60
FW	46	29.0	210/110	100

TABLE IV (Continued)

## BLOOD NITRITE CONCENTRATION IN MAN—SUMMARY

Total Number of Subjects	170
Mean Nitrite in Gamma per 100 cc. Blood	9.45
Standard Deviation	6.25
Standard Error	0.48
Mean Systolic Blood Pressure in mm. Hg	152.7
Standard Deviation	32.4
Standard Error	2.4
Mean Diastolic Blood Pressure in mm. Hg	92.8
Standard Deviation	21.9
Standard Error	1.7
Mean Pulse Pressure in mm. Hg	62.6
Standard Deviation	20.0
Standard Error	1.5
Mean Age in Years	51.4
Standard Deviation	18.7

THE RELATIONSHIP OF THE BLOOD NITRITE LEVEL  
TO BLOOD PRESSURE IN HUMAN SUBJECTS

It has been demonstrated that the administration of nitrites in proper dose is accompanied by a fall in blood pressure, evanescent as the case might be, and that blood normally contains nitrite. The question as to whether the circulating blood nitrite acts physiologically to help control blood pressure presented itself. Is there any difference in the nitrite concentration in the blood of subjects with normal and high blood pressure? In other words, does there exist some correlation between abnormal arterial tension and the blood nitrite level?

TABLE V lists the nitrite levels in the blood of 81 subjects\* in Group I with systolic blood-pressure readings below 145 millimeters of mercury, and diastolic, below 95. Group II (TABLE VI) includes 89 subjects\* selected for high blood pressure, i.e. with readings of 145 millimeters, or over, for systolic and/or 95 millimeters, or over, for diastolic pressure.

The average blood nitrite concentration of the individuals in Group I was 10.49 gamma of nitrite per 100 cc. plus or minus a standard error of 0.68. The mean systolic blood pressure of this group was 122 with a standard deviation of plus or minus 12, and the diastolic pressure was 75.8 plus or minus a standard deviation of 11 millimeters of mercury.

For Group II the average nitrite level was 7.49 plus or minus a standard error of 0.66 gamma per 100 cc. of blood. The average systolic and diastolic pressures were 175 plus or minus a standard deviation of

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\*These patients were obtained for study from the various wards of University Hospital and City Hospitals, Baltimore, Maryland.



27 and 105 plus or minus a standard deviation of 18 millimeters of mercury, respectively.

The mean difference between the blood nitrite level of Group I and that of Group II is 3 gamma which is statistically significant, the standard error of the difference being 0.947.

Recalculation and comparison of the means on the basis of 140 and 85 millimeters of mercury as the hypertensive levels<sup>1</sup> for systolic and diastolic pressures, respectively, or upon the basis of 155 and 100 millimeters of mercury as the respective levels, yields a similar significant difference. TABLE VII sums up the pertinent data for these groupings. It should be noted that these segregating levels do not take into account the fact that the hypertensive level of blood pressure in older subjects is generally higher than that of younger individuals.

When, however, the age factor is considered, i.e. that old individuals tend normally to have higher blood pressures and young have lower blood pressures, the difference between the mean nitrite levels of the normal and hypertensive groups becomes insignificant. TABLE VII shows that the mean difference between the 2 groups is only 1.43 gamma with a standard error of that difference of 0.973. These values are based on the assumption

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<sup>1</sup>There is no general agreement regarding a fixed blood-pressure level which can be considered normal or abnormal. For example, White (1932) considers the upper extreme normal limit in the adult to be 145 and 90 millimeters of mercury for systolic and diastolic pressures, respectively, regardless of age or size. Others give similar views (Morgan 1940, Alvarez and Stanley 1930, Morris, Bazett and McMillan 1927, Hunter 1923). Robinson and Brucer (1939), on the basis of approximately 11,000 normal individuals, give 140 and 90 millimeters for systolic and diastolic blood pressures, respectively, as the levels segregating abnormal blood pressure groups. In their series the average systolic blood pressure for 7,478 men rose from 118 millimeters at 35 years to about 148 millimeters at 85 years of age.

that for individuals 60 to 80 years of age a blood pressure up to 160/95 is the extreme upper normal limit and for individuals 30 years of age or below a normal limiting blood pressure of 135 for systolic and of 85 for diastolic pressure.

TABLE VIII lists the coefficients of correlation between nitrite level and (1) systolic blood pressure, (2) diastolic blood pressure, and (3) pulse pressure, based on the data presented in TABLE IV. All of the coefficients are low and the only one which is statistically significant is that between systolic pressure and nitrite level (-0.24). This table also shows that systolic blood pressure and age are correlated in a positive and highly significant manner. In addition, the nitrite level of the blood is negatively and significantly correlated with age. TABLE IX shows that when subjects with normal blood pressure between the ages of 30 to 59 are compared with hypertensive subjects of the same age range, no significant difference in mean nitrite is observed.

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These authors found the coefficient of correlation between age and systolic pressure for men to be plus 0.14 and that between age and diastolic pressure to be plus 0.22 with a probable error of 0.01. Yet they state that neither of the correlations for men is significant. It should be noted that the chances of such correlations arising through random sampling are very small and therefore the correlations are statistically significant. Another error in their statistical interpretation occurs when they treat their data for females and say "with a standard deviation of 12.3 mm., it seems safe to conclude that the systolic blood pressure of this group of women, regardless of age, ranged from 100 to 125". Plus or minus 1 standard deviation on the normal curve covers only 2/3 of the subjects and therefore 100 to 125 is not the true range. More investigators, however, have reported that higher blood pressures tend to occur normally in older individuals. Saller (1928) studied the blood pressure of 4000 ageing persons and reported normal pressures of 175/93 for male subjects 60-67 years of age, and 186/80 for men 68-89 years of age. Many other investigators have reported the same tendency for the blood pressure to increase with age, although they do not usually give hypertensive levels quite so high (Richter 1925, Kachebries 1933, Thewlis 1941, Cannon 1939, Cohen 1939, Gager 1930, Aaltonen 1939, Lewis 1938, Davis 1930, Riseman and Weiss 1929).

The data thus far tend to show that the nitrite level of the blood of humans is not closely, if at all, associated with abnormal arterial tension. The coefficients of correlation (systolic, diastolic, and pulse pressure) are low or insignificant, and the mean difference of the nitrite levels between normal and hypertensive groups (TABLE VII) is only significant when age is not considered as a factor in dividing the subjects with normal blood pressure from those with high blood pressure. This, coupled with the fact that the coefficients of correlation between age and blood pressure and between age and nitrite level are higher and significant, leads one to suspect that age may be more closely related to the blood nitrite level. General inspection of TABLE IV reveals, in fact, that the older subjects have lower blood nitrite levels than younger individuals and that older individuals make up a considerable part of the hypertensive group. The age factor will be further considered in the next section.

TABLE V

BLOOD NITRITE CONCENTRATION OF  
HUMAN SUBJECTS WITH NORMAL\* BLOOD PRESSURE

Subject	Age	Nitrite in Gamma per 100 cc. Blood	Blood Pressure
1	30	1.0	115/75
2	55	1.0	116/70
3	51	1.0	96/80
4	40	1.0	140/80
5	57	1.0	120/80
6	28	1.0	125/80
7	21	2.0	130/80
8	41	2.5	130/60
9	9	3.0	140/70
10	37	3.0	120/60
11	29	3.0	120/70
12	16	3.0	120/78
13	30	3.0	130/70
14	21	3.0	112/60
15	49	3.0	115/70
16	40	3.0	140/80
17	29	3.0	130/90
18	63	3.0	120/80
19	37	2.0	120/80
20	25	5.0	120/90
21	25	5.0	130/70
22	58	5.0	118/88
23	26	5.0	120/90
24	42	5.0	130/80
25	33	6.0	124/76
26	39	7.0	120/70
27	38	7.0	120/70
28	74	7.0	130/65
29	51	7.0	130/70
30	11	7.0	120/30
31	66	7.0	130/84
32	58	8.0	140/90
33	64	9.0	135/75
34	71	9.0	110/60
35	18	10.0	126/78
36	22	10.0	114/90
37	20	10.0	120/80
38	31	11.0	120/80
39	34	11.0	140/80
40	18	12.0	80/20

TABLE V (Continued)

BLOOD NITRITE CONCENTRATION OF HUMAN  
SUBJECTS WITH NORMAL\* BLOOD PRESSURE

Subject	Age	Nitrite in Gamma per 100 cc. Blood	Blood Pressure
41	59	12.0	130/90
42	36	12.0	120/80
43	28	12.0	110/70
44	12	12.0	120/75
45	40	12.5	125/85
46	49	13.0	120/70
47	27	13.0	120/80
48	23	13.0	134/84
49	58	13.0	140/90
50	59	13.0	140/90
51	38	14.0	118/58
52	40	14.0	90/50
53	40	14.0	130/70
54	32	14.0	128/76
55	25	14.0	128/76
56	30	14.0	140/90
57	18	14.0	120/90
58	25	14.0	110/60
59	25	15.0	122/60
60	69	15.0	80/60
61	58	15.0	140/84
62	50	16.0	120/70
63	36	16.0	130/80
64	20	16.0	130/80
65	63	16.0	122/80
66	44	16.0	130/84
67	18	17.0	140/90
68	25	17.0	114/60
69	20	17.0	105/65
70	23	17.0	128/74
71	58	17.0	118/88
72	58	18.0	132/78
73	26	18.0	110/60
74	21	18.0	110/75
75	32	18.0	130/90
76	30	18.0	122/80
77	35	19.0	116/66
78	37	20.0	124/78
79	45	22.0	130/80
80	52	22.0	125/75
81	64	24.0	138/82

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\*up to 144 mm. Hg systolic and 94 mm. Hg diastolic, regardless of age.

TABLE V (Continued)

BLOOD NITRITE CONCENTRATION OF  
HUMAN SUBJECTS WITH NORMAL BLOOD PRESSURE

Total Number of Subjects	81
Mean Nitrite Level in Gamma per 100 cc. Blood	10.49
Standard Deviation	6.12
Standard Error	0.68
Mean Age, Years	39.6
Standard Deviation	16.0
Mean Systolic Pressure, mm. Hg	121.9
Standard Deviation	11.8
Standard Error	1.3
Mean Diastolic Pressure, mm. Hg	75.8
Standard Deviation	11.2
Standard Error	1.2
Mean Pulse Pressure, mm. Hg	47.9
Standard Deviation	10.0
Standard Error	1.1

TABLE VI

BLOOD NITRITE CONCENTRATION OF HUMAN SUBJECTS WITH HIGH BLOOD PRESSURE

Subject	Age	Nitrite in Gamma per 100 cc. Blood	Blood Pressure
82	76	0.8	150/94
83	81	0.5	150/90
84	91	0.5	204/100
85	55	0.5	180/105
86	41	0.5	180/105
87	80	0.5	180/105
88	53	0.5	150/100
89	43	0.5	250/120
90	64	0.5	190/140
91	54	0.5	160/90
92	60	0.6	210/110
93	65	0.6	210/110
94	72	0.7	200/100
95	75	1.0	164/98
96	72	1.0	160/90
97	76	1.0	150/90
98	71	1.0	150/80
99	72	1.0	250/110
100	50	1.0	205/110
101	55	1.0	200/100
102	66	1.0	190/110
103	78	1.0	174/105
104	56	1.0	222/148
105	71	1.5	170/90
106	67	1.5	180/90
107	69	1.5	200/100
108	65	2.0	230/110
109	39	2.0	140/120
110	54	2.0	160/100
111	73	3.0	200/85
112	68	3.0	165/80
113	46	3.0	160/80
114	61	3.0	150/84
115	24	3.0	190/120
116	45	3.0	150/100
117	57	3.0	170/110
118	54	3.0	155/100
119	71	3.0	180/120
120	70	3.0	190/120
121	41	3.0	145/100
122	52	3.0	125/95

TABLE VI (Continued)

BLOOD NITRITE CONCENTRATION OF  
HUMAN SUBJECTS WITH HIGH\* BLOOD PRESSURE

Subject	Age	Nitrite in Gamma per 100 cc. Blood	Blood Pressure
123	52	7.0	150/58
124	54	7.0	190/90
125	55	7.0	145/90
126	67	7.0	145/95
127	50	7.0	185/100
128	51	7.0	175/135
129	20	7.0	140/100
130	76	7.5	160/100
131	48	9.0	165/100
132	32	9.0	135/95
133	62	10.0	150/80
134	90	10.0	152/84
135	56	10.0	210/110
136	65	10.0	190/100
137	62	10.0	140/100
138	50	10.0	165/105
139	76	10.0	200/120
140	27	11.0	160/90
141	57	11.0	150/90
142	92	11.0	150/90
143	65	11.0	145/95
144	70	11.0	228/110
145	54	11.0	155/90
146	63	11.0	160/100
147	62	11.0	145/100
148	71	12.0	155/95
149	51	12.0	130/98
150	55	12.0	150/105
151	54	13.0	180/80
152	60	13.0	170/90
153	64	13.0	170/115
154	66	13.0	150/110
155	59	13.0	180/120
156	69	14.0	215/95
157	71	14.0	150/88
158	52	14.0	150/110
159	68	14.0	140/100
160	26	15.0	215/180
161	20	15.0	146/46
162	53	16.0	140/100



TABLE VI (Continued)

BLOOD NITRITE CONCENTRATION OF  
HUMAN SUBJECTS WITH HIGH\* BLOOD PRESSURE

Subject	Age	Nitrite in Gamma per 100 cc. Blood	Blood Pressure
163	40	16.0	155/105
164	35	16.0	130/100
165	64	17.0	180/110
166	57	17.0	200/110
167	30	17.0	145/60
168	25	20.0	160/120
169	42	24.0	240/180
170	46	29.0	210/110
Total Number of Subjects			89
Mean Nitrite Level in Gamma per 100 cc. Blood			7.49
Standard Deviation			6.21
Standard Error			0.66
Mean Age, Years			57.7
Standard Deviation			15.2
Mean Systolic Pressure, mm. Hg			172.5
Standard Deviation			27.0
Standard Error			2.8
Mean Diastolic Pressure, mm. Hg			104.5
Standard Deviation			18.4
Standard Error			1.9
Mean Pulse Pressure, mm. Hg			71.2
Standard Deviation			24.8
Standard Error			2.6

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\*systolic, 145 mm. Hg or over; diastolic, 95 mm. or over, regardless of age.

TABLE VII

STATISTICAL ANALYSIS OF DIFFERENCES OF MEAN BLOOD NITRITE  
CONCENTRATION OF NORMAL AND HYPERTENSIVE SUBJECTS  
GROUPED BY VARIOUS SEGREGATING LEVELS

Blood Pressure	No. of Subjects	Mean Nitrite in Gamma per 100 cc. Blood	Diff.	<u>Diff.</u> S.E. of Diff.
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Segregating level regardless of age: 145 mm.  
systolic and/or 95 mm. diastolic

Normal	81	10.49	S.E. 0.68	3.0	3.1
High	89	7.49	S.E. 0.66		

Segregating level regardless of age: 140 mm.  
systolic and/or 85 mm. diastolic

Normal	65	10.86	S.E. 0.79	3.15	3.1
High	105	7.71	S.E. 0.59		

Segregating level regardless of age: 155 mm.  
systolic and/or 100 mm. diastolic

Normal	100	10.03	S.E. 0.54	2.68	2.7
High	70	7.35	S.E. 0.98		

Segregating level: 60 years or over, 160 mm. systolic and/or  
100 mm. diastolic; 30 years or below, 135 mm. systolic and/or  
85 mm. diastolic; all others, 145 mm. systolic and/or 95 diastolic

Normal	91	9.59	S.E. 0.64	1.43	1.4
High	79	8.16	S.E. 0.73		

TABLE VIII

CORRELATION BETWEEN THE (1) BLOOD NITRITE CONCENTRATION AND BLOOD PRESSURE, (2) BLOOD NITRITE CONCENTRATION AND AGE AND (3) BLOOD PRESSURE AND AGE IN HUMAN SUBJECTS.

Association	Coefficient of Correlation (r)	$\frac{r}{\text{Standard Error}}$
Nitrite level and systolic blood pressure	-.24	3.1
Nitrite level and diastolic blood pressure	-.087	1.1
Nitrite level and pulse blood pressure	-.15	1.9
Nitrite level and age	-.31	4.0
Systolic blood pressure and age	plus .384	5.0

Partial Correlation	Coefficient of Partial Correlation	$\frac{r_{12.5}}{\text{Standard Error}}$
Nitrite level and age (blood pressure constant)	-.25	3.2
Nitrite level and systolic pressure (age constant)	-.14	1.8

TABLE IX

THE BLOOD NITRITE CONCENTRATION OF SUBJECTS, 30-59 YEARS OF  
AGE, WITH NORMAL\* AND HIGH BLOOD PRESSURE  
(BASED ON DATA OF TABLE V)

Subjects with normal blood pressure		Subjects with high blood pressure	
Age	Nitrite in Gamma per 100 cc. Blood	Age	Nitrite in Gamma per 100 cc. Blood
55	1.0	55	0.5
51	1.0	41	0.5
40	1.0	53	0.5
57	1.0	43	0.5
41	2.5	54	0.5
37	3.0	50	1.0
30	3.0	55	1.0
49	3.0	58	1.0
40	3.0	39	2.0
37	3.0	54	2.0
58	5.0	46	3.0
42	5.0	43	3.0
33	6.0	57	3.0
39	7.0	54	5.0
38	7.0	41	6.0
51	7.0	52	6.0
58	8.0	52	7.0
31	11.0	54	7.0
54	11.0	35	7.0
59	12.0	31	7.0
36	12.0	48	9.0
40	12.5	32	9.0
49	13.0	56	10.0
58	13.0	50	10.0
59	13.0	57	11.0
38	14.0	54	11.0
40	14.0	31	12.0
40	14.0	55	12.0
32	14.0	54	13.0
30	14.0	39	13.0
58	15.0	52	14.0
50	16.0	53	16.0
38	16.0	40	16.0
44	16.0	35	16.0
58	17.0	57	17.0
58	18.0	30	17.0
32	18.0	42	24.0

TABLE IX (Continued)

THE BLOOD NITRITE CONCENTRATION OF SUBJECTS, 30-59 YEARS OF  
AGE, WITH NORMAL\* AND HIGH BLOOD PRESSURE  
(BASED ON DATA OF TABLE V & VI)

Subjects with normal blood pressure		Subjects with high blood pressure	
Age	Nitrite in Gamma per 100 cc. Blood	Age	Nitrite in Gamma per 100 cc. Blood
30	18.0	46	29.0
33	19.0		
37	20.0		
43	22.0		
52	22.0		
Total number of cases		42	38
Mean Nitrite Level		10.7	8.5
Standard Deviation		6.0	6.8
Standard Error		0.95	1.13
Mean Difference in Nitrite Levels		2.2	
Difference/S.E. of Difference		1.5	

\*Up to 144 mm. Hg systolic and 94 mm. Hg diastolic.

THE RELATIONSHIP OF THE BLOOD NITRITE  
LEVEL TO AGE IN HUMAN SUBJECTS\*

It has been previously demonstrated (TABLE IX) that when subjects with normal blood pressures are compared with hypertensive subjects in the same age range, no significant difference in the mean nitrite concentration of the blood can be observed.

The chi square test (TABLE X) applied to the age-nitrite data for 170 subjects reveals that significance may be attached to the difference in blood nitrite concentration of younger (54 years of age or below) and older subjects (55 years of age and over). This test gives a P between .02 and .01 which means that in only 2 (or less) chances out of 100 can the difference observed occur by chance.

When the subjects are segregated into young and old and their mean blood nitrite levels compared (TABLE XI), the difference also is statistically significant. The difference of 4.25 gamma per 100 cc. between young and old is 4.1 times the standard error of the difference. The odds against the occurrence of a deviation as much as 4 standard errors is great.

An additional series of 32 aged subjects was studied with respect to nitrite concentration of the blood (TABLE XII). The average age for this new group was 73.5 years with a mean nitrite concentration of 5.84 gamma per 100 cc. blood (standard deviation 2.0 and standard error 0.58). With 1 or 2 exceptions, these subjects had blood pressures within normal limits. Here is further indication that low nitrite levels are not necessarily associated with high arterial tension.

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\*These subjects were obtained for study from the chronic wards of the Baltimore City Hospitals.

The difference between the mean value (11.43) for the blood nitrite of subjects below 40 years of age (TABLE XI) and that (5.84) of the new group of 52 aged individuals is 5.59 gamma per 100 cc. This difference is highly significant, the difference being over 7 times greater than its standard error. TABLE XIII summarizes the statistical analysis for all subjects below 40 and above 60 years of age (data of TABLES XI and XII combined). The coefficient of correlation ( $r$ ) for the entire group of 202 subjects, calculated by the Pearson product moment method, is  $-0.36$  (standard error 0.07). Figure 1 indicates graphically in a general way the relationship between the blood nitrite and age.

The true explanation for the lower average nitrite level in older individuals is not yet forthcoming. In the aged the blood sugar tends to become elevated. According to Marshall (1931) the renal threshold rises from 170-180 milligrams per cent in young adults to 200-210 milligrams per cent in the healthy aged. Porter and Langley (1926) reported the basal glucose level of fasting persons tends to rise with age. Perhaps the disordered carbohydrate metabolism in these individuals with resultant increase in a reducing agent effects a lowered blood nitrite level. Analysis of the blood of several diabetic subjects, however, revealed no significant decrease in nitrite concentration.

Various intestinal bacteria have the property of reducing nitrates to nitrites (Stieglitz and Palmer 1936, Zinsser and Bayne-Jones 1928). Although there is no evidence available, it may be that the intestinal flora necessary for this reduction is diminished in septuagenarians and octogenarians. These nitrate-reducing bacteria, according to Stieglitz and Palmer (1936), are more active in a semisolid medium than in a solid

medium. Alvarez (1924) also points out that bacteria generally grow in the colon where the feces are liquid enough to furnish a proper medium. Increase of the fluidity of the colonic contents, he states, often results in a subsequent increase in bacterial count and drying out of the feces in the colon is associated with a reduction in organisms. Perhaps in older individuals the state of the intestinal contents make for a more solid medium and a decrease in bacteria or bacterial activity.

As will be further discussed in the following pages, the work of other investigators has revealed that the reduction of nitrates to nitrite in the body appears to be a part of a physiological oxidation-reduction system, especially prevalent in liver tissue (Bernheim and Dixon 1928). A diminution in enzymatic activity of this type may occur in ageing, although such evidence has not yet been revealed by investigators interested in geriatrics.

Finally, it must be admitted that the older subjects selected for this study were patients in the chronic wards of Baltimore City Hospitals, institutionalized in most cases because of senility. The diet provided for these individuals may differ in certain nutritional respects from that available to other ageing persons.



TABLE X

CHI SQUARE TEST APPLIED TO AGE AND NITRITE LEVEL  
IN THE BLOOD — DATA OF TABLE IV

Nitrite Level in Gamma per 100 cc.	Observed Number ( $f_o$ )	Expected Number ( $f$ )	$\frac{(f_o-f)^2}{f}$	
0 - 5.9	Young*	30	37.2	1.39
	Old	33	25.8	2.00
6 -11.9	Young	22	24.2	.20
	Old	19	16.8	.28
12 and over	Young	47	39	1.64
	Old	19	27	2.40

\*54 years of age and under

Chi Square	7.91
N	2
P	Less than .02

TABLE XI

THE AGE FACTOR: THE BLOOD NITRITE CONCENTRATION OF  
SUBJECTS THIRTY NINE YEARS OF AGE OR UNDER COMPARED  
WITH THOSE SIXTY YEARS OF AGE OR OVER

Subjects 39 years of age or under		Subjects 60 years of age or over	
Age	Nitrite in Gamma per 100 cc. Blood	Age	Nitrite in Gamma per 100 cc. Blood
28	1.0	76	0.5
39	2.0	81	0.5
9	3.0	91	0.5
37	3.0	60	0.5
29	3.0	64	0.5
16	3.0	60	0.6
30	3.0	65	0.6
21	3.0	72	0.7
29	3.0	75	1.0
37	3.0	90	1.0
39	2.0	72	1.0
24	3.0	76	1.0
25	5.0	71	1.0
25	5.0	72	1.0
26	5.0	66	1.0
33	6.0	78	1.0
35	7.0	71	1.5
31	7.0	67	1.5
20	7.0	68	1.5
39	7.0	65	2.0
38	7.0	73	3.0
11	7.0	68	3.0
18	10.0	71	5.0
32	9.0	70	5.0
22	10.0	61	3.0
20	10.0	67	7.0
31	11.0	74	7.0
27	11.0	66	7.0
18	12.0	76	7.5
36	12.0	64	9.0
28	12.0	71	9.0
12	12.0	62	10.0
31	12.0	90	10.0
39	13.0	65	10.0
27	13.0	62	10.0
23	13.0	76	10.0
38	14.0	92	11.0
32	14.0	65	11.0
25	14.0	70	11.0

TABLE XI (Continued)

THE AGE FACTOR: THE BLOOD NITRITE CONCENTRATION OF  
SUBJECTS THIRTY NINE YEARS OF AGE OR UNDER COMPARED  
WITH THOSE SIXTY YEARS OF AGE OR OVER

Subjects 39 years of age or under		Subjects 60 years of age or over	
Age	Nitrite in Gamma per 100 cc. Blood	Age	Nitrite in Gamma per 100 cc. Blood
30	14.0	63	11.0
18	14.0	62	11.0
25	14.0	71	12.0
25	15.0	60	13.0
26	15.0	64	13.0
20	15.0	66	13.0
36	16.0	69	14.0
20	16.0	71	14.0
35	16.0	68	14.0
18	17.0	63	16.0
25	17.0	69	15.0
20	17.0	64	17.0
23	17.0	63	3.0
30	17.0	64	24.0
26	18.0		
21	18.0		
32	18.0		
30	18.0		
33	19.0		
25	20.0		
37	20.0		
Total number of cases		60	53
Mean Nitrite Level		11.43	7.2
Standard Deviation		5.16	5.67
Mean Difference in Nitrite Levels		4.23	
Difference/S.E. of Difference		4.1	

TABLE XII

THE AGE FACTOR: THE BLOOD NITRITE CONCENTRATION OF  
ADDITIONAL AGED SUBJECTS

Age	Blood Pressure	Nitrite in Gamma per 100 cc. Blood
63	150/90	2
63	120/72	2
75	140/74	2
74	110/82	3
80	140/68	4
76	122/88	4
70	140/70	4
73	150/70	4
61	116/78	5
77	115/65	5
76	150/90	5
72	180/100	6
79	135/70	6
96	140/80	6
79	140/82	6
79	138/74	6
75	120/70	6
73	150/90	6
87	145/95	6
70	160/100	6
67	130/78	6
72	130/70	7
73	150/70	7
68	102/60	7
82	190/92	7
67	130/70	8
84	110/76	8
70	150/70	8
73	150/80	8
63	110/65	8
72	150/90	9
62	140/75	10
Total Number of Subjects		32
Mean Age in Years		73.5
Mean Nitrite Level		5.8 gamma per 100 cc. blood
Standard Deviation		2.0
Standard Error		0.38

TABLE XIII

SUMMARY OF STATISTICAL ANALYSIS FOR THE AGE-NITRITE  
RELATIONSHIP IN HUMAN SUBJECTS

	Subjects below 40 years of age	Subjects* 60 years of age or over	Difference	<u>Difference</u> S.E. of Diff.
Total No. Subjects	60	85		
Mean Age	27	71		
Mean Nitrite per 100 cc. Blood	11.43	6.41	5.02	5.9
Standard Error	0.67	0.51		

\*Subjects of TABLES XI and XII combined.

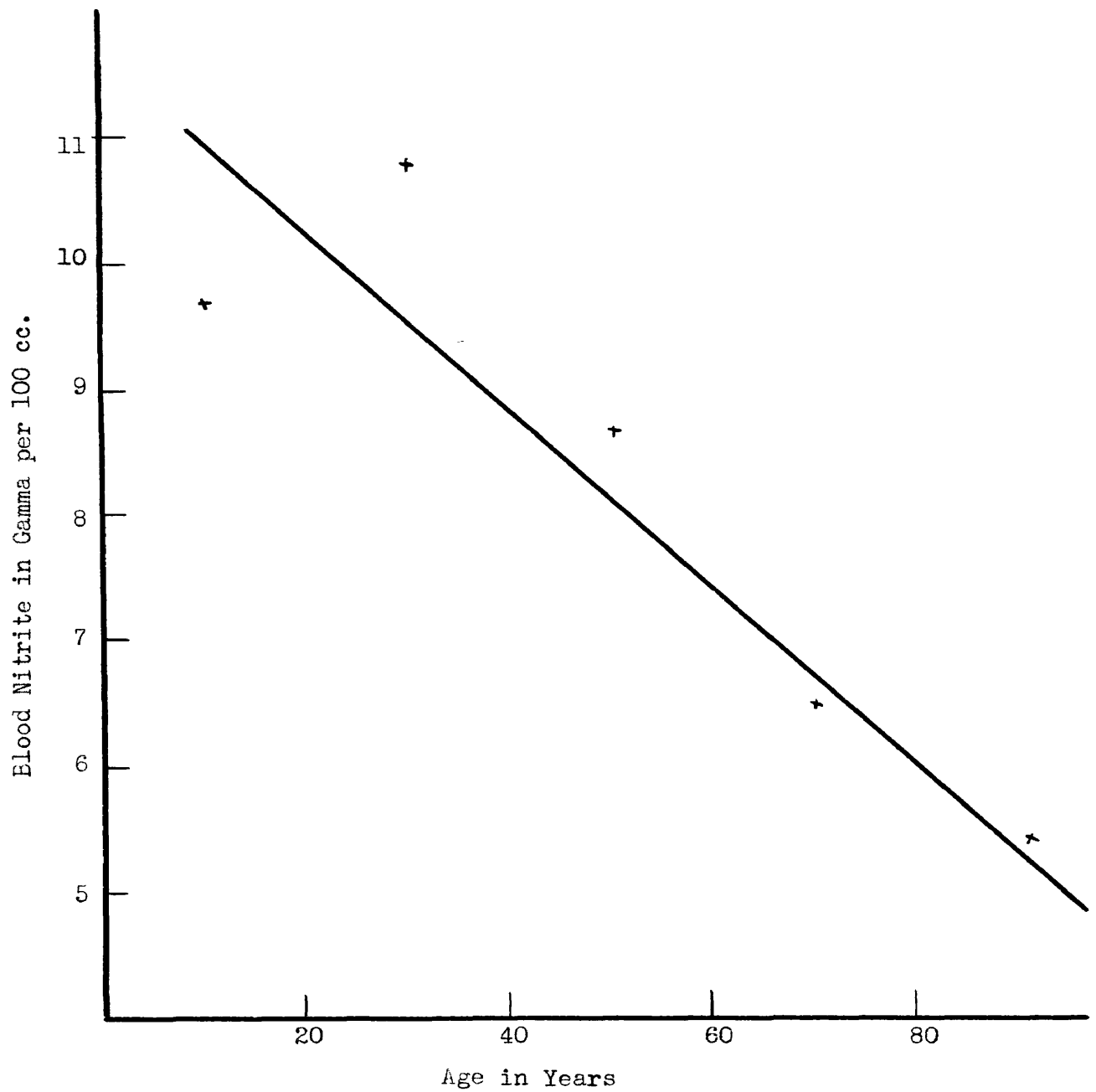


FIGURE 1. THE RELATIONSHIP BETWEEN AGE AND BLOOD NITRITE CONCENTRATION IN HUMAN SUBJECTS (202)

THE INFLUENCE OF OTHER FACTORS UPON THE CONCENTRATION  
OF THE BLOOD NITRITE

From the foregoing, ageing apparently is associated with a decrease in the blood nitrite concentration. An investigation to determine what other factors influence this level was executed.

Human Subjects. No difference in the blood nitrite level between male and female or between white subjects and negroes was noted. Individuals with diabetes possessed a nitrite level within the normal range. Nor was any trend in the blood nitrite concentration observed with different non-protein nitrogen levels of the blood. Patients with glomerular nephritis and high NPN values possessed normal or low nitrite levels. Blood withdrawn without stasis (no tourniquet or pressure around the arm) was no different from blood obtained with stasis as regards to nitrite concentration.

Dogs. Various substances were either mixed with the daily food ration or fed by stomach tube to dogs.

1. Amino acids. A ready source of the amino group and nitrogen was supplied in the form of gelatin and glycine in daily doses of 30 grams and 2 grams, respectively. These compounds did not significantly affect the blood nitrite, even after feeding for 6 to 7 days.

2. Ammonium chloride. This salt was administered to determine the effect of the ammonia as well as the acidotic action. In doses of 10 grams daily for 10 days ammonium chloride did not alter the nitrite level. The slight change in the acid-base balance (this was not measured) does not destroy nitrite, nor is nitrogen in the form of ammonia converted to nitrite. The possibility remains, however, that if such two tendencies do exist, each component may counteract the other, resulting in no net effect.

3. Potassium nitrate. The convertability of inorganic nitrate to nitrite by the body was studied in several animals. Daily doses of 5 to 15 grams for 5 to 6 days resulted in no increase at the end of the feeding period. A dose of 35 grams dissolved in 60 cc. of water was administered by stomach tube to 2 dogs (weighing 10 and 7.5 kilograms). The blood nitrite was followed for 1, 3, 6, and 24 hours after feeding. No increase in nitrite of the blood resulted. Furthermore, no symptoms of nitrite toxicity (chocolate-colored blood, cyanosis, weakness, dyspnea, etc.) could be observed.

4. Fasting. Analysis of the blood of 5 dogs fasted for 8 to 16 days revealed no change in nitrite as compared with the control values.

5. Organic nitrates. Erythrol tetranitrate, isomannide dinitrate and glyceryl trinitrate, given orally in human therapeutic doses did not raise the blood nitrite concentration; doses as high as 3 grams, 1 gram and 5 cc., respectively, raised the blood-nitrite level but slightly (by 6 to 10 gamma per 100 cc.) several hours after feeding.

6. Sodium nitrite. After oral doses as small as 120 milligrams, the blood-nitrite level rose from 12 to 65 gamma per 100 cc. within 40 minutes after feeding. Two hours after administration of the drug, the nitrite level returned to its original value. Experiments with sodium nitrite and the organic nitrates will be presented in greater detail and further discussed in a later section of the thesis.

In summary, attempts to alter the blood-nitrite level by several methods in dogs met with failure. These methods included prolonged fasting and feeding amino acids, ammonium chloride, potassium nitrate. Sodium nitrite alone readily raised the blood-nitrite level. Feeding experiments in humans were not conducted but (aside from the ageing process) stasis, increased



blood sugar and non-protein levels, color, sex, and arterial tension were not related to the nitrite concentration of the blood. Stieglitz and Palmer (1937) observed that dextrose, in vitro, destroys nitrite and that no nitrite, in contrast to the findings of the author, could be detected in the blood of several diabetic patients.

#### DISCUSSION

Seekles and Sjollem (1932) reported that 100 to 200 grams of potassium nitrate suddenly introduced into the rumen of an ox caused symptoms of poisoning and claim that 10 per cent of the nitrate administered was reduced to nitrite in the animal's paunch. This nitrite, according to these workers, changes the oxyhemoglobin of the blood to methemoglobin and is itself converted to nitrate. Williams and Hines (1940) administered 80 grams of potassium nitrate as a drench to sheep. Within 8 hours the animal died and the blood picture revealed a considerable conversion of hemoglobin to methemoglobin. More recently, Bradley and coworkers (1940) observed that following the feeding of a particular lot of oat hay containing about 5 per cent potassium nitrate, cattle developed methemoglobinemia and died. Horses and sheep, as well, occasionally suffered from "oat hay" poisoning. These investigators conclude, since nitrite rather than nitrate was known to form methemoglobin, that the nitrate must have been converted to nitrite in the digestive tract.

As Maynard (1941) points out, it appears that "oat hay poisoning" is of most concern in the case of ruminants because possibly of a special opportunity for chemical changes provided by their digestive tracts. He

adds that no cases have been reported in omnivora. As previously stated, no increase in nitrite resulted after administration of large doses of nitrate to dogs in this laboratory.

Hiatt (1940), however, observed that oral administration of sodium nitrate in comparatively small doses to dogs caused obvious methemoglobinemia. He presumes the nitrate is converted into nitrite by intestinal bacteria and cites the work of Keith, Whelan and Bannick (1930) and Tarr (1933). These authors do not state, however, that administration of nitrates results in the ready liberation of nitrite. The first group of investigators gave 10 grams of ammonium nitrate daily to patients and animals without toxic effects. Total doses of 560 grams were administered during hospital treatment without symptoms. A small number of patients with nephritis or nephrosis developed cyanosis and methemoglobinemia. One case with marked retention of nitrates for several days indicated that nitrates in considerable amounts may be widely distributed throughout the body without giving rise to gross methemoglobinemia or cyanosis. Interestingly, a massive dose of ammonium nitrate in one patient caused a rise in blood pressure from 150/100 to 200/125 which remained elevated for 10 days. Quoting from the paper by Keith and coworkers (1930),

"These observations support the view that the toxemia was caused by the nitrate ion".

"\_\_\_\_\_systemic nitrite-like actions did not occur, with the exception of the rare development of methemoglobinemia".

"Thus, the reduction of nitrates to nitrites rarely occurs in animals".

Tarr (1933) treated many patients with ammonium nitrate. Six to 8 grams were administered orally per day for periods as long as 18 weeks. In

only 4 patients was transient methemoglobin observed (confirmed spectroscopically) but the author points out that renal dysfunction was present in these subjects prior to medication.

#### CONSIDERATION OF THE POSSIBLE SOURCES AND PHYSIOLOGICAL ROLE OF THE BLOOD NITRITE

Evidence that the oral administration of large amounts of nitrate is followed by appreciable rise in blood nitrite is lacking. But this does not preclude the possibility that nitrite is formed in minute quantities during some physiological process involving the tissue nitrate (Whelan (1935) has reported an average of 1 milligram of nitrate nitrogen is present per gram of tissue), that it enters the circulation wherein it is maintained at a fairly constant concentration. A survey of the literature has revealed a number of findings from various branches of biology and chemistry which bear on this problem and which may have not been heretofore collected and correlated.

Various types of foods contain nitrates. Numerous organisms present in the normal intestinal flora are capable of reducing nitrate to nitrite, especially *B. coli* and *lactis-aerogenes* (Zobell 1932, Stieglitz and Palmer 1936, Aibel 1937, Aibel and coworkers 1937). Some bacteria oxidize ammonia to nitrite (Zinsser and Bayne-Jones 1928) and various intestinal strains destroy nitrite by converting it to nitrate (Zobell 1932 and Stieglitz and Palmer 1936).

A number of investigators have found that plant enzymes convert nitrate to nitrite. They believe that plants containing nitrates yield nitrites by action of both plant and animal enzymes (Kastle and Elvove 1904, Bernheim

and Dixon 1928).

Animal tissues also apparently contain systems capable of reducing nitrates to nitrites. Stepanow (1902) showed that tissues which normally contained no nitrite were able to form it when ground with nitrates. This same author, studying the tissues of rabbits and dogs, found nitrite in the white matter of the brain, small intestines, medullary substance of the kidney, lymph glands but not in the grey matter of the brain, liver, cortex of the kidney, muscle and blood. Stepanow does not describe the method used for analyzing for nitrite in blood.

Bernheim and Dixon (1928) reported that the livers of the sheep, pig, rabbit, dog, chicken, rat and guinea pig reduce nitrates very readily and that the liver appears to be the main seat of this phenomenon. They demonstrated by in vitro experiments that nitrate is reduced during the process of oxidation of aldehyde, xanthine, adenine by oxidases of liver tissue. The amount of nitrite produced per time unit in the presence of different substances was exactly parallel to the rate of reduction of methylene blue. In other words the nitrate readily takes the place of methylene blue as hydrogen acceptor in other oxidation-reduction systems (Dixon and Thurlow 1924, Bach 1911, Openheimer and Stern 1939). It is interesting to note that Kastle and Elvove in 1911 found that alcohols and aldehydes also greatly accelerate the reduction of sodium nitrate by plant extracts. At that time they suspected that nitrates play a vital role in biochemical oxidations and reductions.

Aubel (1937) believes that nitrates are important in the mechanism of biological oxidations and that they constitute under certain processes of dehydrogenation in cellular metabolism, along with oxygen, the only acceptors of hydrogen. Baudisch (A 1921) discovered that reduction of

alkali nitrate to nitrite can be complete by iron in the absence of oxygen. Nascent hydrogen, he states, is not involved because iron is capable of drawing to itself the subsidiary valence of the nitrate oxygen atom and consequently of abstracting an atom of oxygen from the nitrate molecule. Baudisch (B 1921) also reported that dextrose and iron are factors in the reduction of tissue nitrate to nitrite.

To recapitulate, the source of the normal blood nitrite may possibly be from the

- (1) ingestion of food containing the nitrite ion,
- (2) reduction of nitrate in the intestines by bacteria or plant enzymes,
- (3) reduction of nitrate in the tissues or blood stream as part of an oxidation-reduction system.

Apparently there is a continuous liberation and concomitant disappearance of nitrite from the blood stream, resulting in a fairly constant level of blood nitrite. Noteworthy is the finding that the elevated nitrite concentration after intravenous or oral administration of sodium nitrite quickly returns to its original level in the blood stream. It may be oxidized to nitrate (Krantz, Carr and coworkers 1938). Apparently the kidneys do not remove nitrite for the bladder urines of several dogs were tested for nitrite at intervals after injection of sodium nitrite with negative results. Nitrates injected into the circulation of dogs also disappear rapidly (Crandall et al. 1929) but here a large percentage of the nitrate is probably eliminated by the kidneys.

THE NITRITE CONCENTRATION IN THE BLOOD AFTER ADMINISTRATION  
OF SODIUM NITRITE AND SEVERAL ORGANIC NITRATES TO DOGS

A survey of the literature has revealed that nearly all of the research work purporting to show that the organic nitrates liberate nitrite ions in order to reduce blood pressure was performed before the turn of the present century (Atkinson 1888, Leech 1893, Bradbury 1895, Marshall 1897). Accurate methods for quantitative analysis of nitrite in the blood had not yet been evolved and it appears that investigators based their views on gross and spectroscopic changes in the blood i.e., methemoglobin formation.

On the basis of these earlier studies most pharmacology texts up to the present time have stated that the organic nitrates depend for their action upon hydrolysis and the liberation of nitrite and, hence, have included in their discussions of "nitrites" both the nitrites and the nitrates.

Krantz, Carr and coworkers (1940) have recently demonstrated that after erythrol tetranitrate, glyceryl trinitrate and mannitol hexanitrate are heated with sodium hydroxide to bring about hydrolysis they do not retain depressor activity in the dog. Isomannide dinitrate is an exception in that it retains this activity after an attempt is made to hydrolyze it by alkali treatment. In vitro experiments showed that the latter compound is refractory to hydrolysis and this fact offers a possible explanation for the above phenomena, i.e., why the depressor activity of isomannide dinitrate, treated by heat and sodium hydroxide and then administered, is not diminished. The other 3 organic nitrates, on the other hand, are readily hydrolyzable by the same chemical means.

As Krantz and Carr (1940) point out in their report, there remains the unlikely possibility of the immediate reduction of the intact nitrate

ester to the nitrite ester, the latter being responsible for the depressor effect observed.

An investigation was undertaken to shed light on the following interrelated problems:

A. Intravenous Administration

- (1) the fate of sodium nitrite when injected into the blood stream,
- (2) the amount of detectable nitrite which must be present in the blood before a fall in blood pressure can obtain,
- (5) a further study of the question as to whether the organic nitrates, erythrol tetranitrate, isomannide dinitrate and glyceryl trinitrate are converted into nitrite in the body before causing a reduction in blood pressure after intravenous administration, and

B. Oral Administration

- (1) to what extent the nitrite level of the blood is increased after oral administration of sodium nitrite and the organic nitrates.

A - Intravenous Administration. Under nembutal anesthesia (0.75 cc. of a 4 per cent solution per kilogram), sodium nitrite and the organic nitrates employed were administered to dogs intravenously. The blood pressure in the carotid artery was measured and recorded by kymograph in the usual manner. Injections were made into the cannulated saphenous or femoral vein and samples of blood were withdrawn from the exposed contralateral femoral artery. Solutions of erythrol tetranitrate, isomannide dinitrate

and glyceryl trinitrate were prepared in concentrations ranging from 1:10,000 to 1:100 in 20 to 35 per cent alcohol. The usual dosage of the nitrates was 0.75 cc. per kilogram. A quantity of alcohol equivalent to that present in each of the doses the dogs received did not exert any depressor activity. The dosage of sodium nitrite ranged from 0.75 cc. of a 1:1000 solution to 0.3 cc. of a 5 per cent solution per kilogram.

#### Sodium Nitrite

Intravenous administration to 4 dogs of 0.3 cc. of 5 per cent sodium nitrite solution per kilogram (TABLE XIV) resulted in an average fall in blood pressure of 35 millimeters of mercury (26 per cent below original) within 1 minute after injection. The nitrite concentration in gamma per 100 cc. of blood at this time was, on the average, 740, this level decreasing to 184,107 and 68 gamma after 5 minutes, 15 minutes and 45 minutes, respectively. It is of interest to note that of the nitrite found at the time (within 1 minute) of depressor activity, 75 per cent had disappeared 5 minutes after injection and only another 15 per cent during the next 40 minutes.

TABLE XV, which is based on the above data, demonstrates that only an average of 7 per cent of the amount of nitrite injected was recoverable from the blood obtained at the time of the fall in blood pressure, and from that withdrawn 45 minutes after injection less than 1 per cent could be recovered. The number of milligrams of nitrite present in 100 cc. of blood, assuming that all the nitrite remained in the blood stream, was calculated from the estimated blood volume and from the amount of nitrite injected. This theoretical amount was considered 100 per cent of the nitrite theoretically recoverable from the blood instantly after adminis-



tration of the drug. The total amount of blood in the dog was calculated on the basis of 97 cc. per kilogram body weight (Meek and Gasser 1918). In each case the control value for the blood before administration of the drug was subtracted from the actual nitrite level to obtain "recovered nitrite".

Evidently the level of the nitrite concentration does not bear a direct relationship to the blood pressure after the initial fall has ensued. Even after most of the injected nitrite had disappeared from the blood stream the blood pressure remained considerably below normal. Figure 1 illustrates graphically the rate of disappearance of the injected nitrite and the relationship between the nitrite and blood pressure levels.

The explanation for the disappearance of nitrite has not yet been definitely determined. Possibly, the nitrite ion is quickly oxidized to nitrate (Krantz, Carr et al. 1940) in the blood stream, or perhaps it leaves the vascular system to enter the tissues to become converted into ammonia (Harnack, cited by Solis-Cohen and Githens 1928) or is removed by the liver.

Sodium nitrite was intravenously administered to several animals (TABLE XVI) in small enough dosages to evoke no or little fall in blood pressure. Under the influence of a nitrite level of 90 gamma per 100 cc. of blood there was no depressor effect (dogs numbered 35 and 36), but when a 5 to 7 per cent fall in blood pressure occurred the blood nitrite concentration was relatively high (several hundred gamma per 100 cc. in dogs numbered 32, 33 and 34). As with higher dosages of sodium nitrite, the detectable nitrite after injection of small dosages disappeared rapidly from the blood stream even when no fall in blood pressure was observed

(TABLE XVII). The results of the experiments with sodium nitrite tend to bear out the findings in human blood studies previously reported where no relationship between the blood nitrite concentration and arterial tension could be observed.

#### Organic Nitrates

The effects of injecting the organic nitrates upon the blood pressure and nitrite concentration of the blood are presented in TABLES XVIII, XIX, and XX.

In concentrations of 1:1000, neither erythrol tetranitrate, isomannide dinitrate nor glyceryl trinitrate increased the blood nitrite level at the time of average blood pressure falls of 44, 22, and 40 per cent, respectively. In the case of isomannide dinitrate, a very slight increase (2 to 3 gamma per 100 cc. blood) was noted about 17 minutes after injection of the drug when the blood pressure had risen appreciably.

Erythrol tetranitrate, in relatively high concentration of 1:100, effected an average of 50 per cent fall in blood pressure in 4 dogs with no increase in blood nitrite at the time of fall. Five minutes after injection there was an average increase of 9 gamma per 100 cc., but sixty minutes after injection the nitrite level returned to its original value. There were slight increases over normal (ranging from 1 to 18 gamma with an average of 6 gamma per 100 cc.) at the time of fall in blood pressure with isomannide dinitrate and glyceryl trinitrate in 1:100 concentrations. The highest level of nitrite in the blood after injecting any of these organic nitrates was but a very small fraction of the lowest nitrite level observed with a comparable fall in blood pressure with sodium nitrite. Furthermore, the increase in nitrite concentration (TABLE XVI) under

sufficiently minute doses of sodium nitrite to cause no fall in blood pressure was considerably greater than any increase in nitrite occurring after injection of the organic nitrates with appreciable depression of the blood pressure.

Finally, erythrol tetranitrate (TABLE XVIII) and glyceryl trinitrate (TABLE XX) in small doses of 0.75 cc. of 1:10,000 solutions per kilogram lowered the blood pressure 14 per cent and 41 per cent, respectively, with no increase in the blood nitrite over the control value. In several other dogs, not listed in these tables, erythrol tetranitrate and glyceryl trinitrate in the same dosage produced a fall of over 30 per cent in blood pressure.

The unlikely possibility remained that from this small quantity of organic nitrate sufficient nitrite might have been quickly liberated in the body to effect a hypotensive action, i.e., be liberated and disappear so quickly as to escape detection by the chemical analysis employed. The amount of nitrate ( $\text{NO}_3$ ) contained in 0.75 cc. of a 1:10,000 solution of either erythrol tetranitrate or glyceryl trinitrate is 0.06 milligram. This amount of nitrate administered per kilogram of dog could give rise to 0.045 milligram of nitrite ( $\text{NO}_2$ ) per 100 cc. of blood, if 100 per cent of the nitrate were converted. Crandall (1929), however, showed that 20 per cent of glyceryl trinitrate injected intravenously into dogs could be recovered from the blood 1 minute after administration of the drug. Hence, if 80 per cent of the nitrate were converted and none left the circulatory system to enter the tissues, a maximum of 0.038 milligram of nitrite, or 38 gamma, per 100 cc. of blood might have been produced, if such rapid hydrolysis and reduction were possible. Such a minute amount of nitrite

in the blood, however, could not possibly effect a reduction in blood pressure. This is illustrated by the experimental findings with sodium nitrite reported above, and also by mathematical deduction. The amount of sodium nitrite required to theoretically place a concentration of 38 gamma of nitrite ion per 100 cc. in the blood stream immediately upon injection is 0.75 cc. of a 1:15,000 solution per kilogram. This theoretical dose is 150 times less than the actual amount of sodium nitrite required to produce a fall in blood pressure comparable to that effected by erythrol tetranitrate or glyceryl trinitrate in the concentration under discussion. In fact nitrite levels of the magnitude of 38 gamma per 100 cc. could not be associated with a fall in blood pressure at all.

In summary, under the conditions of these experiments, the organic nitrates apparently do not reduce the blood pressure of dogs when administered intravenously by first liberating nitrite because of the following considerations:

- (1) no significant increase in the nitrite concentration of the blood over normal could be detected after their administration by the analytical method employed,
- (2) no fall in blood pressure was observed after injection of sodium nitrite in amounts which raised the nitrite level of the blood considerably, and
- (3) the nitrates were capable of depressing the blood pressure when injected in minute doses of 0.75 cc. of 1:10,000 per kilogram. From this amount can not be liberated sufficient nitrite to account for the

hypotensive activity observed even if most of the nitrate were hydrolyzed and reduced at the time of the fall in blood pressure.

B - Oral Administration. Sodium nitrite and the organic nitrates employed in the previous experiments are administered therapeutically to man by the oral or sublingual route. The possibility that some reaction occurs within the alimentary tract, enabling the liberation and absorption of sufficient nitrite from the nitrates into the circulatory system to bring about the therapeutic effect, still remained. The following experiments were devised to study this problem.

Human therapeutic doses, as well as massive doses, of several compounds were given per os either by stomach tube or gelatin capsule to a series of fasted dogs. Blood samples were withdrawn from the external jugular vein just prior to feeding the drug and at varying intervals of time after administration.

TABLE XXI shows that nitrite in the form of the sodium salt is absorbed and, in the human therapeutic dose, disappears from the blood stream about 2 hours after oral administration. A total dose of 1.5 grams of sodium nitrite killed a dog within 45 minutes (Dog No. 71). The blood nitrite concentration associated with a lethal intravenous dose of sodium nitrite was found to be in several dogs, 2 or more milligrams per 100 cc. of blood. A dose of 250 milligrams (Dog No. 40) produced such symptoms as labored breathing, weakness, listlessness and semiprostration approximately 45 minutes after feeding. At this time the blood nitrite level was 280 gamma per 100 cc. Two hours later the animal appeared its normal self again.

Neither erythrol tetranitrate nor isomannide dinitrate in doses of 2 to 5 grains appreciably increased the blood nitrite. After large doses of the latter drug (0.5 to 1.0 gram), the nitrite level (Dogs numbered 38 and 39) increased about 30 gamma per 100 cc. 2 hours after administration. Based on the acute intravenous experiments described before, this concentration of nitrite in the blood is apparently not sufficient to account for any hypotensive activity. Erythrol tetranitrate even in dosage as high as 3 grams (about 50 times greater than the human therapeutic dose) did not effect an increase in blood nitrite concentration.

Glyceryl trinitrate, freshly prepared and administered in the comparatively huge dose of 5 cc., caused the death of an animal in approximately 4 hours. No appreciable increase in the nitrite concentration of the blood resulted.

Noteworthy was the absence of the characteristic chocolate color of methemoglobin in the blood of the dogs receiving the nitrates, whereas the dark brown coloration of the blood was definitely observed even after comparatively small oral doses of sodium nitrite. Analysis for methemoglobin was not performed in these experiments.

In summary, neither the intravenous nor oral administration of the organic nitrates, erythrol tetranitrate, isomannide dinitrate and glyceryl trinitrate, results in the ready liberation of the nitrite ion to the blood. The basis for the general opinion that these compounds yield nitrite in the body before they act to lower the blood pressure has rested chiefly on the observation of "methemoglobin" formation after their administration (since nitrite produces methemoglobin).

It should be pointed out that the above conclusions are founded upon experiments with dogs. There remains the possibility that the reactions of dogs to nitrates differ from those of other animal species. The validity, however, of the earlier reports that methemoglobin was actually produced in man and laboratory animals after nitrate administration is open to question. As Goodman and Gilman (1941) point out, the production of methemoglobin by sodium nitrite can readily be demonstrated by the addition of this compound to shed blood, whereupon the characteristic chocolate color and spectrographic absorption bands can be detected. Van Slyke and Vollmund (1925) found that nitrite penetrates the red cells instantly and forms methemoglobin within them. Goodman and Gilman (1941) add, however, that when nitroglycerin is added to blood, in vitro, a similar change does not occur.

The uncertainty of the presence of methemoglobinemia in early reports after nitrate therapy is attributable to the fact that the first specific quantitative test for methemoglobin was apparently not proposed until 1911 (Van Slyke 1925). Many of the statements of methemoglobinemia were made because of the presence of cyanosis in subjects receiving nitrates or because of a brown coloration of the blood. Spectroscopic examination of blood for methemoglobin may not have been entirely accurate according to Kobert (1906) who stated that the ordinary spectroscopic examination is uncertain when less than 25 per cent of the blood pigment is in the form of methemoglobin. Rabe (1919) pointed out inconsistencies in the results reported by different authors from spectroscopic examinations. As for cyanosis, this condition accompanied by change of part of the hemoglobin into a non-oxygen carrying form, may not be due to methemoglobin formation at

all (Loeb, Bock and Fitz 1921). Furthermore, recent investigations by Krantz, Carr and collaborators (1938) have demonstrated that under full depressor response of isomannide dinitrate, glyceryl trinitrate or erythrol tetranitrate, blood taken from the carotid artery of the dog under ether anesthesia contained no methemoglobin and showed no characteristic spectral band when examined in the Keuffel and Esser Color Analyzer. Tests were conducted at intervals until one hour after injection. In vitro experiments were also conducted with one per cent dilutions of normal dog blood saturated with erythrol tetranitrate, mannitol hexanitrate and isomannide dinitrate, respectively. The presence of methemoglobin in the blood could not be detected when tested by the Color Analyzer one hour later.

Hence, the conclusions of earlier investigators that nitrite must be formed from nitrates before the blood pressure can be reduced are, to the mind of the author, founded on an insecure basis. Further research is required for the elucidation of the hypotensive action by the organic nitrates.



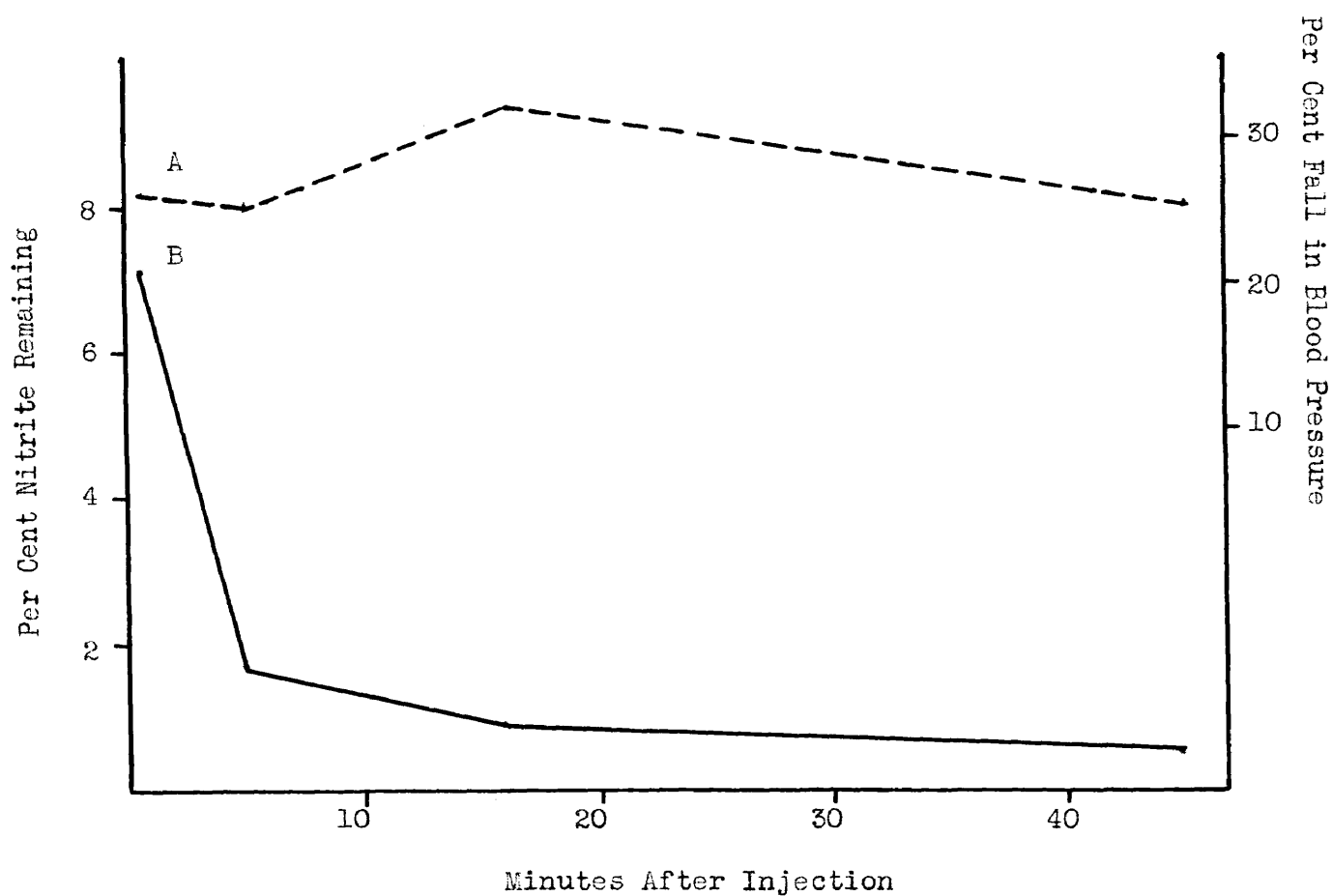


FIGURE 2. THE PERCENTAGE FALL IN BLOOD PRESSURE AND RATE OF DISAPPEARANCE OF NITRITE AFTER INTRAVENOUS ADMINISTRATION IN DOGS

Curves A and B show, respectively, the average percentage fall in blood pressure and the percentage of nitrite remaining in the blood stream of 4 dogs following the intravenous injection of 15 milligrams of sodium nitrite per kilogram.

TABLE XIV

EFFECT OF SODIUM NITRITE ADMINISTERED INTRAVENOUSLY UPON THE BLOOD PRESSURE OF DOGS AND THE CONCENTRATION OF NITRITE IN THE BLOOD AT INTERVALS AFTER INJECTION

Dog	Time After Injection	Blood Pressure	Millimeters Below Original	Percentage Fall	Nitrite in Gamma per 100 cc. Blood
1	0	132	0	0	12
	45 secs.	96	46	34	870
	5 min.	102	30	22	290
	15 min.	82	50	36	160
	45 min.	86	46	34	100
2	0	100	0	0	10
	48 secs.	78	22	22	750
	5 min.	68	32	32	250
	15 min.	62	38	38	180
	45 min.	60	40	40	110
3	0	184	0	0	10
	42 secs.	140	44	23	1,120
	5 min.	138	46	25	68
	15 min.	124	60	32	38
	45 min.	154	30	16	22
4	0	110	0	0	12
	40 secs.	72	38	34	220
	5 min.	84	26	23	130
	20 min.	84	26	23	49
	45 min.	94	16	14	40
AVERAGE					
4 (total no.)	0	131	0	0	11
	45 secs.	96	35	26	740
	5 min.	98	33	25	184
	15 min.	88	43	32	107
	45 min.	98	33	25	68

Dose: 0.3 cc. of 5% sodium nitrite per kilogram body weight

TABLE XV

RECOVERABLE NITRITE AND RATE OF DISAPPEARANCE OF NITRITE FROM BLOOD OF DOGS AFTER INTRAVENOUS ADMINISTRATION OF SODIUM NITRITE: BASED ON DATA OF TABLE XIV

Dog No.	Wt. Kg.	Time After Injection	Per Cent Fall in Blood Pressure	Mg. Nitrite Recovered per 100 cc. Blood	Per Cent Recovered	Per Cent Disappearance (Cumulative)
1	9.4	Immediately	0	10.5*	100.0	0
		45 secs.	34	0.86	8.3	91.7
		5 min.	22	0.28	2.7	97.5
		15 min.	36	0.15	1.4	98.6
		45 min.	34	0.09	0.87	99.13
2	9.0	Immediately	0	10.5*	100.0	0
		48 secs.	22	0.74	7.1	92.9
		5 min.	32	0.24	2.3	97.7
		15 min.	38	0.17	1.6	98.4
		45 min.	40	0.10	0.97	99.03
3	11.0	Immediately	0	10.5*	100.0	0
		48 secs.	23	1.11	10.7	89.3
		5 min.	25	0.058	0.56	99.44
		15 min.	32	0.028	0.27	99.73
		45 min.	16	0.012	0.12	99.88
4	13.3	Immediately	0	10.5*	100.0	0
		40 secs.	34	0.208	2.0	98.0
		5 min.	23	0.118	1.1	98.9
		20 min.	23	0.037	0.36	99.64
		45 min.	14	0.028	0.27	99.73
AVERAGE ( NOS. 1 to 4 INCLUSIVE )						
		Immediately	0	10.5*	100.0	0
		45 secs.	26	0.73	7.1	92.9
		5 min.	25	0.175	1.7	98.3
		16 min.	32	0.096	0.93	99.07
		45 min.	25	0.057	0.55	99.45

\* Calculated

TABLE XVI

INTRAVENOUS ADMINISTRATION OF SODIUM NITRITE IN AMOUNTS CAUSING NO OR LITTLE FALL IN BLOOD PRESSURE IN DOGS AND THE CONCENTRATION OF NITRITE IN THE BLOOD AT INTERVALS AFTER INJECTION

Dog No.	Time After Injection	Blood Pressure	Millimeters Below Original	Percentage Fall	Nitrite in Gamma per 100 cc. Blood
32 *	0	104	0	0	10
	19 secs.	98	6	5	320
	5 min.	84	20	19	150
	15 min.	82	22	21	120
	45 min.	92	12	11	70
33 *	0	184	0	0	12
	60 secs.	170	14	7	1,110
	3.5 min.	144	40	21	750
	5 min.	150	34	18	500
	15 min.	140	44	23	200
	45 min.	148	36	19	150
34 **	0	154	0	0	10
	27 secs.	146	8	5	1,000
	5 min.	150	4	2.5	400
	15 min.	150	4	2.5	350
35 ***	0	118	0	0	10
	45 secs.	118	0	0	90
	5 min.	120	0	0	40
36 x	0	124	0	0	5
	45 secs.	124	0	0	55
	7 min.	116	8	6	27

Sixty Minutes After First Injection

y	0	106	0	0	5
	45 secs.	106	0	0	33
	5 min.	108	0	0	12

\* Dose: 0.75 cc. of 1:100 solution per kilogram

\*\* Dose: 0.50 cc. of 1:100 solution per kilogram

\*\*\* Dose: 0.75 cc. of 1:500 solution per kilogram

x Dose: 0.75 cc. of 1:750 solution per kilogram

y Dose: 0.75 cc. of 1:1,000 solution per kilogram

TABLE XVII

RECOVERABLE NITRITE AND RATE OF DISAPPEARANCE OF NITRITE FROM BLOOD  
OF DOGS AFTER INTRAVENOUS ADMINISTRATION OF SMALL AMOUNTS OF SODIUM  
NITRITE: BASED ON DATA OF TABLE XVI

Dog No.	Wt. Kg.	Time After Injection	Per Cent Fall in Blood Pressure	Mg. Nitrite Recovered per 100 cc. Blood	Per Cent Recovered	Per Cent Disappearance (Cumulative)
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Dose: 0.75 cc. of 1:100 solution per kilogram

32	9.7	Immediately	0	5.2*	100	0
		19 secs.	5	0.31	5.9	94.1
		5 min.	19	0.14	2.7	97.3
		15 min.	21	0.11	2.1	97.9
		45 min.	11	0.06	1.1	98.9

Dose: 0.75 cc. of 1:100 solution per kilogram

53	14.3	Immediately	0	5.2*	100	0
		60 secs.	7	1.09	20.9	79.1
		3.5 min.	21	0.74	14.2	85.8
		5 min.	18	0.49	9.4	90.6
		15 min.	23	0.19	3.6	96.4
		45 min.	19	0.14	2.7	97.3

Dose: 0.5 cc. of 1:100 solution per kilogram

34	14.3	Immediately	0	3.4*	100	0
		27 secs.	5	0.99	29.1	70.9
		5 min.	2.5	0.39	11.4	88.6
		15 min.	2.5	0.34	10.0	90.0

Dose: 0.75 cc. of 1:500 solution per kilogram

35	11.9	Immediately	0	1.03*	100	0
		45 secs.	0	0.08	7.7	92.3
		5 min.	0	0.03	2.9	97.1

TABLE XVII (Continued)

RECOVERABLE NITRITE AND RATE OF DISAPPEARANCE OF NITRITE FROM BLOOD  
OF DOGS AFTER INTRAVENOUS ADMINISTRATION OF SMALL AMOUNTS OF SODIUM  
NITRITE: BASED ON DATA OF TABLE

Dog No.	Wt Kg.	Time After Injection	Per Cent Fall in Blood Pressure	Nitrite Mg. Recovered per 100 cc. Blood	Per Cent Recovered	Per Cent Disappearance (Cumulative)
Dose: 0.75 cc. of 1:750 solution per kilogram						
36	6.6	Immediately	0	0.87*	100	0
		45 secs.	0	0.05	7.4	92.6
		7 min.	8	0.03	4.4	95.8
Dose: 0.75 cc. of 1:1000 solution per kilogram						
		Immediately	0	0.52*	100	0
		48 secs.	0	0.028	5.3	94.7
		5 min.	0	0.007	1.3	98.7

\*Calculated

TABLE XVIII

EFFECT OF ERYTHROL TETRANITRATE IN VARYING DOSAGES ADMINISTERED INTRAVENOUSLY UPON THE BLOOD PRESSURE OF DOGS AND THE CONCENTRATION OF NITRITE IN THE BLOOD AT INTERVALS AFTER INJECTION

Dog No.	Time After Injection	Blood Pressure	Millimeters Below Original	Percentage Fall	Nitrite in Gamma per 100 cc. Blood
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Dose: 0.75 cc. of 1:10,000 solution per kilogram

5	0	142	0	0	8
	40 secs.	122	20	14	8
	5 min.	134	8	5	8
	20 min.	132	10	7	8

Dose: 0.75 cc. of 1:1,000 solution per kilogram

6	0	160	0	0	10
	45 secs.	80	80	50	12
	5 min.	118	42	26	10
	15 min.	112	48	30	12
	45 min.	130	30	17	12
7	0	190	0	0	12
	45 secs.	108	82	43	12
	5 min.	174	16	8	10
	15 min.	174	16	8	11
	30 min.	172	18	9	11
8	0	200	0	0	12
	42 secs.	110	90	45	12
	5 min.	166	34	17	10
	15 min.	184	16	8	12
	30 min.	180	20	10	12
9	0	136	0	0	12
	60 secs.	80	56	41	12
	5 min.	116	20	14	10
	15 min.	120	16	11	10
	45 min.	122	14	10	10
	60 min.	118	18	13	10

AVERAGE (DOGS NOS. 6 to 9 INCLUSIVE)

0	172	0	0	11.5
48 secs.	95	77	44	12
5 min.	144	28	16	10
15 min.	148	24	15	11
45 min.	151	21	12	11

TABLE XVIII (Continued)

EFFECT OF ERYTHROL TETRA-NITRATE IN VARYING DOSAGES ADMINISTERED  
INTRAVENOUSLY UPON THE BLOOD PRESSURE OF DOGS AND THE CONCENTRA-  
TION OF NITRITE IN THE BLOOD AT INTERVALS AFTER INJECTION

Dog No.	Time After Injection	Blood Pressure	Millimeters Below Original	Percentage Fall	Nitrite in Gamma per 100 cc. Blood
Dose: 0.75 cc. of 1:100 solution per kilogram					
10	0	180	0	0	15
	15 secs.	80	100	55	15
	5 min.	158	22	12	17
	15 min.	168	12	6	17
	45 min.	170	10	5	18
11	0	164	0	0	4
	48 secs.	72	92	56	5
	5 min.	96	68	41	7
	15 min.	118	48	29	8
	45 min.	156	8	5	5
	60 min.	154	10	6	5
12	0	180	0	0	18
	100 secs.	80	100	55	26
	5 min.	76	104	57	44
	15 min.	74	106	58	28
	45 min.	98	82	45	17
	60 min.	108	72	40	18
13	0	170	0	0	8
	60 secs.	116	54	31	8
	5 min.	126	44	25	12
	15 min.	110	60	35	10
	45 min.	112	58	34	8
	60 min.	112	58	34	8
AVERAGE ( NOS. 10 to 13 INCLUSIVE)					
	0	174	0	0	11
	56 secs.	87	87	50	12
	5 min.	114	60	34	20
	15 min.	118	56	32	15
	45 min.	134	40	23	12
	60 min.	125	49	28	10



TABLE XIX

EFFECT OF ISOMANNIDE DINITRATE IN VARYING DOSAGES ADMINISTERED  
INTRAVENOUSLY UPON THE BLOOD PRESSURE OF DOGS AND THE CONCENTRA-  
TION OF NITRITE IN THE BLOOD AT INTERVALS AFTER INJECTION

Dog No.	Time After Injection	Blood Pressure	Millimeters Below Original	Percentage Fall	Gamma of Nitrite in 100 cc. Blood
Dose: 0.75 cc. of 1:1,000 solution per kilogram					
14	0	124	0	0	10
	38 secs.	104	20	16	10
	5 min.	110	14	11	10
	22 min.	104	20	16	10
	45 min.	110	14	11	12
15	0	124	0	0	5
	43 secs.	88	36	29	5
	5 min.	120	4	3	5
	15 min.	124	0	0	8
16	0	130	0	0	9
	29 secs.	106	24	18	8
	5 min.	110	20	15	8
	15 min.	106	24	18	8
	30 min.	100	30	23	9
17	0	176	0	0	8
	30 secs.	134	42	23	8
	5 min.	190	-14	-8	10
	15 min.	160	16	9	10
	30 min.	164	12	7	12
AVERAGE ( NOS. 14 to 17 INCLUSIVE )					
	0	139	0	0	8
	35 secs.	108	31	22	8
	5 min.	153	6	4	8
	17 min.	124	15	11	12
	35 min.	125	14	11	11

TABLE XIX (Continued)

EFFECT OF ISOMANNIDE DINITRATE IN VARYING DOSAGES ADMINISTERED  
INTRAVENOUSLY UPON THE BLOOD PRESSURE OF DOGS AND THE CONCENTRA-  
TION OF NITRITE IN THE BLOOD AT INTERVALS AFTER INJECTION

Dog No.	Time after Injection	Blood Pressure	Millimeters Below Original	Percentage Fall	Nitrite in Gamma per 100 cc. Blood
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Dose: 0.75 cc. of 1:100 solution per kilogram

18	0	142	0	0	10
	35 secs.	86	56	39	12
	5 min.	138	4	2	10
	15 min.	124	18	12	10
	45 min.	143	0	0	12
19	0	96	0	0	12
	45 secs.	44	52	54	20
	5 min.	54	42	43	12
	15 min.	56	40	41	12
	45 min.	66	24	25	12
20	0	172	0	0	8
	40 secs.	96	76	44	9
	5 min.	118	54	31	15
	15 min.	134	38	22	10
	45 min.	136	36	20	10
21	0	126	0	0	12
	38 secs.	70	56	44	22
	5 min.	110	16	12	28
	15 min.	98	28	22	28
	45 min.	104	22	17	44
22	0	162	0	0	4
	30 secs.	72	90	55	6
	5 min.	130	32	19	8
	15 min.	140	22	30	10
	45 min.	150	12	7	6

AVERAGE ( NOS. 18 to 22 INCLUSIVE )

0	140	0	0	9
38 secs.	74	66	47	14
5 min.	110	30	21	15
15 min.	110	30	21	14
45 min.	120	20	14	17

TABLE 17

EFFECT OF GLYCERYL TRINITRATE IN VARYING DOSAGES ADMINISTERED  
INTRAVENOUSLY UPON THE BLOOD PRESSURE OF DOGS AND THE CONCENTRATION OF NITRITE IN THE BLOOD AT INTERVALS AFTER INJECTION

Dog No.	Time After Injection	Blood Pressure	Millimeters Below Original	Percentage Fall	Nitrite in Gamma per 100 cc. Blood
Dose: 0.75 cc. of 1:10,000 solution per kilogram					
23	0	140	0	0	5
	40 secs.	82	58	41	5
	5 min.	136	4	3	5
	15 min.	140	0	0	5
	45 min.	136	4	3	5
Dose: 0.75 cc. of 1:1,000 solution per kilogram					
24	0	142	0	0	5
	60 secs.	82	60	42	5
	5 min.	126	16	11	5
	15 min.	150	12	8	5
	45 min.	126	16	11	5
25	0	182	0	0	8
	45 secs.	110	72	39	8
	5 min.	158	24	13	8
	15 min.	162	20	10	8
	45 min.	156	26	14	10
26	0	182	0	0	8
	45 secs.	108	74	40	8
	5 min.	170	12	7	8
	15 min.	164	18	9	8
	45 min.	144	38	20	9
27	0	168	0	0	5
	48 secs.	106	62	36	5
	5 min.	156	12	7	5
	15 min.	154	14	8	5
	45 min.	152	16	9	5

TABLE XX (Continued)

EFFECT OF GLYCERYL TRINITRATE IN VARYING DOSAGES ADMINISTERED  
INTRAVENOUSLY UPON THE BLOOD PRESSURE OF DOGS AND THE CONCENTRATION OF NITRITE IN THE BLOOD AT INTERVALS AFTER INJECTION

Dog No.	Time After Injection	Blood Pressure	Millimeters Below Original	Percentage Fall	Nitrite in Gamma per 100 cc. Blood
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AVERAGE ( NOS. 24 to 27 INCLUSIVE )

	0	169	0	0	7
	40 secs.	102	67	40	7
	5 min.	153	16	9	7
	15 min.	153	18	9	7
	45 min.	145	24	14	7

Dose: 0.75 cc. of 1:100 solution per kilogram

28	0	178	0	0	7
	60 secs.	178	100	56	25
	5 min.	136	42	23	56
	15 min.	128	50	27	40
	45 min.	158	40	22	40
29	0	196	0	0	5
	48 secs.	108	88	44	8
	5 min.	156	40	20	8
	15 min.	146	50	25	8
	45 min.	150	66	33	12
30	0	128	0	0	5
	50 secs.	44	84	65	10
	5 min.	80	98	76	13
	15 min.	72	56	43	8
	45 min.	86	42	32	6
31	0	167	0	0	6
	45 secs.	79	88	53	10
	5 min.	117	50	30	12
	15 min.	120	47	28	10
	45 min.	125	42	25	10

AVERAGE ( NOS. 28 to 31 INCLUSIVE )

	0	167	0	0	6
	51 secs.	77	90	53	13
	5 min.	110	57	34	22
	15 min.	117	50	29	17
	45 min.	120	47	28	17

TABLE XXI

EFFECT OF ORAL ADMINISTRATION OF SODIUM NITRITE AND  
SEVERAL ORGANIC NITRATES UPON THE NITRITE LEVEL  
IN THE BLOOD OF DOGS

Dog No.	Weight Kg.	Time After Administration	Nitrite Level in Gamma per 100 cc. Blood
Sodium Nitrite: Dose 120 mg.			
42	7.9	0	12
		38 min.	65
		70 min.	40
		138 min.	12
Sodium Nitrite: Dose 250 mg.			
40	6.5	0	10
		48 min.	280
		78 min.	150
		148 min.	60
		17 hr.	10
Sodium Nitrite: Dose 500 mg.			
70	9	0	8
		20 min.	8
		40 min.	8
		60 min.	285
Sodium Nitrite: Dose 1.5 g.			
71	8.5	Dead 43 minutes after administration	
Erythrol Tetranitrate: Dose 150 mg.			
57	9.8	0	15
		50 min.	15
		2 hr.	15
		3 hr.	15

TABLE XXI (Continued)

EFFECT OF ORAL ADMINISTRATION OF SODIUM NITRITE AND  
SEVERAL ORGANIC NITRATES UPON THE NITRITE LEVEL  
IN THE BLOOD OF DOGS

Dog No.	Weight Kg.	Time After Administration	Nitrite Level in Gamma per 100 cc. Blood
Erythrol Tetranitrate: Dose 300 mg.			
39	10	0	8
		20 min.	8
		40 min.	8
		60 min.	8
Erythrol Tetranitrate: Dose 1 g.			
74	9.8	0	6
		20 min.	6
		30 min.	6
		60 min.	6
		90 min.	17
		120 min.	12
		150 min.	10
Erythrol Tetranitrate: Dose 3 g.			
41	7.6	0	7
		45 min.	7
		90 min.	7
		3 hr.	9
		7 hr.	7
Isomannide Dinitrate: Dose 200 mg.			
37	10	0	6
		20 min.	6
		60 min.	6
		100 min.	10
		120 min.	6

TABLE XXI (Continued)

EFFECT OF ORAL ADMINISTRATION OF SODIUM NITRITE AND  
SEVERAL ORGANIC NITRATES UPON THE NITRITE LEVEL  
IN THE BLOOD OF DOGS

Dog No.	Weight Kg.	Time After Administration	Nitrite Level in Gamma per 100 cc. Blood
Isomannide Dinitrate: Dose 300 mg.			
73	5.6	0	6
		20 min.	6
		110 min.	8
		150 min.	6
Isomannide Dinitrate: Dose 500 mg.			
74	9.8	0	8
		45 min.	8
		100 min.	10
		160 min.	8
		220 min.	8
		280 min.	8
Isomannide Dinitrate: Dose 500 mg.			
39	7.5	0	8
		40 min.	8
		75 min.	8
		155 min.	38
Isomannide Dinitrate: Dose 1 g.			
38	8	0	10
		40 min.	10
		60 min.	12
		90 min.	18
		120 min.	38

TABLE XXI (Continued)

EFFECT OF ORAL ADMINISTRATION OF SODIUM NITRITE AND  
SEVERAL ORGANIC NITRATES UPON THE NITRITE LEVEL  
IN THE BLOOD OF DOGS

Dog No.	Weight Kg.	Time After Administration	Nitrite Level in Gamma per 100 cc. Blood
Glyceryl Trinitrate: Dose 5 cc.			
37	10	0	8
		7 min.	8
		20 min.	8
		35 min.	8
		42 min.	8
		75 min.	14
		150 min.	10
		Died 4 hours after administration	



THE EFFECT OF SODIUM NITRITE UPON THE ARTERIAL BLOOD  
PRESSURE OF UNANESTHETIZED HYPERTENSIVE RATS.

According to Grollman and his associates (1940), nitrites and nitrates do not appreciably lower arterial blood pressure. An increase in cardiac output, they state, compensates for the dilatation in the peripheral circulation and it is only when this stimulation of cardiac action fails that a fall in blood pressure ensues. It is maintained that in the case of anesthetized animal the blood pressure decline is probably conditioned by this failure of the heart to respond due to the disordered state of circulation induced by the anesthetic. In order to obviate any possible influence of anesthesia upon blood pressure, the experiments to be described presently were performed on the unanesthetized animal.

Measurement of Blood Pressure - The systolic blood pressures in the tails of the rats were determined by the plethysmographic method of Williams, Harrison and Grollman (1939). The drug was not administered until a fairly constant level of blood pressure from day to day had established itself. The blood pressures of normal unoperated rats ranged between 75 and 110 millimeters of mercury, the mode being approximately 100. The variation from day to day for each rat, after sufficiently trained, was not more than 5 to 10 millimeters. In the experiments to be presented, each recording represents the average of 2 successive blood pressure readings varying in most cases not more than 0 and 5 millimeters from each other.

Operative Technique - Rats were rendered hypertensive by (1) subtotal nephrectomy (Chanutin and Ferris 1932) or (2) bilateral wrapping of cellophane of the kidneys or (3) unilateral nephrectomy combined with unilateral

cellophane wrapping, according to the method of Page (1939). The last two procedures were found superior because the operation can be performed in 1 step and a lower mortality rate results than with subtotal nephrectomy.

The perinephritis caused by application of cellophane to the kidneys resulted in the formation of a fibrocollagenous hull, as observed at necropsy, which constricted the renal parenchyma but avoided the pedicle. This compression of renal tissue in the scar supposedly produces the desired ischemia and resultant hypertension. According to Page (1939), sufficient evidence has been collected to suggest that the physiological mechanism responsible for the hypertension is similar to that concerned when hypertension results from constricting the renal arteries by the Goldblatt clamp. More recently, Page (1940) has demonstrated that renin is liberated into the renal vein in increased amounts from kidneys of dogs made hypertensive by cellophane perinephritis. Produced by the method of subtotal nephrectomy, the elevated blood pressure in rats apparently is not dependent upon the production of renal ischemia, as evidenced by the renal-flow investigations of Dock and Rytand (1937).

Only about half of the animals became hypertensive and it took from 2 to 6 months for hyperplasia to develop. In the majority of the hypertensive rats the duration of the elevated arterial tension was limited; i.e., about 6 months after the peak had been reached the blood pressure had fallen considerably.

Histopathology - Sections of kidneys obtained from rats treated by the cellophane method showed a pathological picture ranging from mild acute nephritis to renal necrosis following application of the cellophane for 3 to 8 months. More specifically, these changes included:

in glomeruli: proliferation and swelling of epithelium; hyaline changes; subcapsular adhesions; engorgement; abnormal numbers of polymorphonuclear leucocytes.

in tubules: dilatation; hyaline casts.

in interstitial tissue: fibrosis and calcified bodies.

in arterioles: hyaline changes and medial hypertrophy.

Results of Administering Sodium Nitrite - Inspection of TABLE XXII, which presents the results with a non-toxic, oral dose of 4 milligrams of sodium nitrite per 100 grams of body weight, reveals that the blood pressure can be reduced by this drug. Twelve experiments were performed on 5 rats. The difference between the average blood pressure before oral administration (140 millimeters) and the lowest average blood pressure (118 millimeters, 30 minutes after feeding) was 22 millimeters of mercury. With the standard error of the difference equal to 3.1 millimeters, this difference is statistically significant.

TABLE XXIII is concerned with 12 experiments on the intraperitoneal injection of sodium nitrite (2.5 milligrams per 100 grams of body weight) into 4 rats. An average reduction of 24 millimeters about 20 minutes after injection was observed. Statistical analysis shows that the standard error of the difference between the average original blood pressure (140 millimeters) and the average blood pressure 20 minutes after injection (116 millimeters) is 6.4 millimeters.

Figure 3 shows graphically the average reductions of blood pressure after oral and intraperitoneal administration of sodium nitrite, based on the data of TABLES XXII and XXIII.

No symptoms of nitrite poisoning were observed throughout the experiments.

By oral administration, a dosage as high as 15 milligrams per 100 grams was apparently not sufficient to evoke abnormal signs but 20 or more milligrams per 100 grams was toxic or lethal. Intraperitoneal doses of 10 milligrams or more were followed by toxic symptoms or death.

Grollman and coworkers (1940) reported that inappreciable reductions in blood pressure were induced in hypertensive rats when sodium nitrite was given in a dosage of 0.1 gram per rat per day mixed with the animal's food. Although this method of administration may effect a rather uniform and constant supply of the drug, it is conceivable that so little of the nitrite was ingested or available for absorption into the blood stream at any given time that a hypotensive action could not obtain.

In summary, sodium nitrite, when administered properly and in adequate dosage, reduces the arterial blood pressure of unanesthetized hypertensive rats.

TABLE XXII

THE EFFECT OF ORAL ADMINISTRATION OF SODIUM NITRITE ON THE  
BLOOD PRESSURE OF UNANESTHETIZED HYPERTENSIVE RATS

Blood Pressure in mm Hg

Rat No.	Minutes After Administration of 4 mg per/grams <sup>100</sup>								
	0	10	20	30	45	60	90	120	180
1	138	138	114	116	124	127	135	135	138
2	140	95	95	102	95	100	100	105	125
3	145	134	124	122	140	138	140	140	140
4	141	135	140	137	133	136	134	134	140
Average	141	126	119	119	123	125	127	129	136
1	140	118	128	115	134	135	135	138	142
2	140	98	100	105	100	100	95	100	110
3	142	80	125	118	117	130	122	130	143
4	141	125	128	128	125	138	143	144	152
Average	141	105	115	114	119	126	124	128	137
1	135	132	130	130	122	125	120	120	140
5	140	130	120	110	114	105	110	110	120
3	136	130	124	115	125	140	133	138	138
4	145	130	133	130	140	140	155	140	134
Average	139	131	127	121	125	128	130	127	138
<u>Total Average</u>	140	120	122	118	122	126	127	128	137

TABLE XXIII

THE EFFECT OF INTRAPERITONEAL INJECTION OF SODIUM NITRITE ON  
THE BLOOD PRESSURE OF UNANESTHETIZED HYPERTENSIVE RATS

Blood Pressure in mm. Hg

Rat No.	Minutes After Administration of 2.5 mg. per 100 grams								
	0	10	20	30	45	60	120	180	480
Experiment 1									
6	135	141	135	135	95	112	115	132	144
7	140	120	100	109	90	80	108	136	140
8	133	133	104	110	70	110	110	130	130
4	148	142	135	133	145	136	150	130	135
Average	139	134	118	122	100	110	121	132	137
Experiment 2									
6	146	128	135	128	133	110	123	125	135
7	153	128	135	116	129	124	122	110	130
8	144	120	80	80	106	100	115	120	130
4	143	140	124	139	140	135	125	132	148
Average	147	129	119	116	127	117	121	122	136
Experiment 3									
6	135	105	90	108	108	102	128	125	133
7	130	113	108	102	116	118	129	128	124
8	139	125	112	104	112	108	140	145	143
4	143	135	142	127	128	130	130	130	155
Average	137	120	113	110	116	115	132	132	139
Total Average	140	126	116	116	114	114	125	129	137

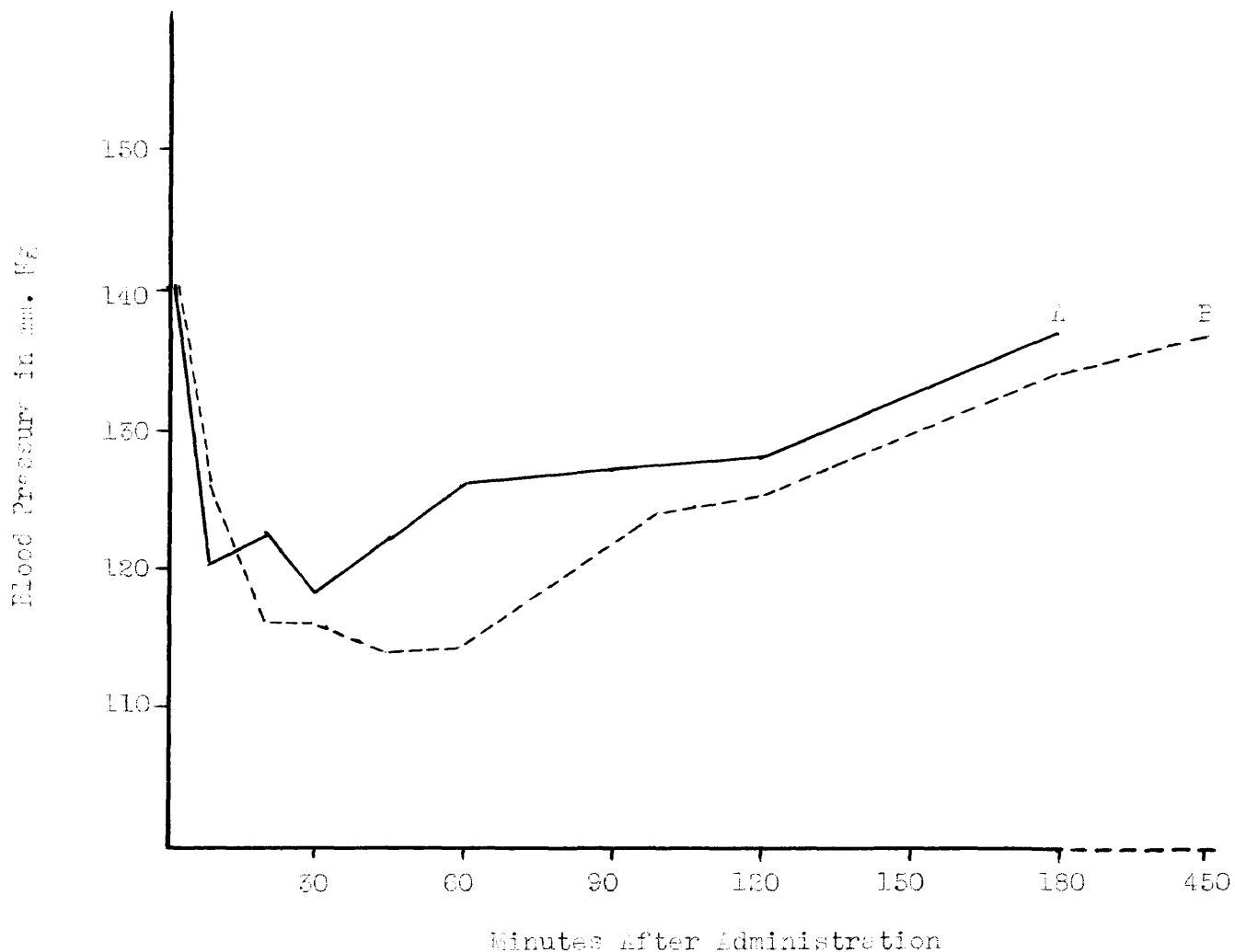


FIGURE 3. THE EFFECT OF ORAL AND INTRAPERITONEAL ADMINISTRATION OF SODIUM NITRITE UPON THE BLOOD PRESSURE IN UNANESTHETIZED HYPERTENSIVE RATS

Curves A and B show, respectively, the average fall in blood pressure after oral administration of 4 milligrams of sodium nitrite per 100 grams and intraperitoneal injection of 2.5 milligrams of the same drug per 100 grams body weight (based upon data of TABLES XXII and XXIII).

## SUMMARY AND CONCLUSIONS

1. A simple method for the quantitative determination of nitrite in blood is described herein.
2. The blood of the dog, monkey, steer, and man contains approximately 10 gamma of nitrite per 100 cc.
3. In a group of 202 subjects there was apparently a tendency for the blood-nitrite concentration to decline with ageing. Insignificant or no association with non-protein nitrogen of the blood or arterial tension was found.
4. Attempts to influence the level of blood nitrite in dogs by feeding nitrates, gelatin, glycine, and ammonium chloride met with failure. The oral administration of sodium nitrite readily elevated the blood-nitrite concentration.
5. The possible sources and physiological role of the blood nitrite are discussed.
6. Further evidence has been accumulated to demonstrate that, contrary to commonly heretofore-accepted views, the organic nitrates may act to reduce the blood pressure without first being converted into nitrite in the body. The experiments from which this conclusion is derived were performed on dogs.
7. To obtain the smallest reduction in blood pressure following intravenous injection of sodium nitrite, the nitrite concentration of the blood in the dog must be increased 8 to 10 times the normal level.
8. Following parenteral and oral administration of sodium nitrite, nitrite disappears rapidly from the blood stream.



9. After the initial fall in blood pressure evoked by sodium nitrite, the blood pressure of the dog apparently does not depend on the level of the blood nitrite; i.e., the hypotensive effect of the drug endures even after almost all of the nitrite has disappeared from the blood stream.
10. Administered orally or parenterally, sodium nitrite in non-toxic dosage lowers the systolic blood pressure in the unanesthetized hypertensive rat.

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