LVIII. BLOOD AMMONIA¹

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I. INTRODUCTION

CONCERNING the normal content of free NH_3 in human venous blood, it was previously shown [Conway, 1935] that the characteristic value of this was either zero or below the analytical level (less than 1 part in 30 millions) but that when blood was shed into an open vessel a rapid rise in the free NH_3 content occurred in the first 5 min. after shedding.² Observations of the same kind are described in the present paper on the rabbit (ether anaesthesia) and the fowl.

The formation and origin of NH_3 after shedding is not only of interest in relation to deamination in the blood stream itself, but also by comparison with similar processes in tissues. This blood NH_3 is here described under three headings, namely, the "alpha" NH_3 which originates immediately after shedding and for which some evidence is given that it may arise from adenosine (or possibly from minute concentrations of free adenylic acid), the "beta" NH_3 which is shown to derive after a series of stages from adenylypyrophosphoric acid, and the "gamma" NH_3 which appears to come either from vegetable adenylic acid or from adenyldoxyribonucleotide or alternatively from substances liberating these in plasma and in the red corpuscles. The "beta" and "gamma" moieties correspond to the B and A fractions of Heller & Klisiecki [1932].

From the evidence presented it would appear that most if not all the blood NH_3 forming in sterile blood collected by open shedding derives finally from adenosine.³ This is not necessarily so for the fowl's blood which can also deaminate guanosine, cytidine and even adenine freely. Neither does it hold for mammalian blood maintained at pH 7.4 by suitable CO_2 tension. The "beta" NH_3 then derives very largely from adenylic acid, only part of the NH_3 appearing via adenosine.

Concerning the specificity of the deamination in blood a large number of substances containing amino (or volatile amine) groups has been studied for possible deamination in blood, but no other substances besides adenosine or associated compounds have been found to yield NH_3 (or amines) at appreciable rates or at rates comparable with the normal formation of ammonia in shed blood. Thus we have repeatedly found that arginine, if added in small amounts to shed rabbit's blood or buffered after addition at a pH between 7 and 8, only gives negative results. It may be noted, however, that the main point in view was to ascertain the origins of the blood NH_3 and that the conditions of the investigation did not differ very widely from the normal.

¹ A preliminary report of this work appeared in *Nature*, **139**, 1937, and **142**, 1938.

² Results on the normal dog similar to these findings for the human subject have been reported to us by H. Kropowski, the Pathological Laboratory, Warsaw University.

³ The presence of adenosine deaminase in plasma was shown by one of us [Cooke, 1936] prior to Drury's communication to the Physiological Society.

II. THE NORMAL CONTENT OF NH_3 IN CIRCULATING BLOOD*Methods*

Experimental. The blood either from an arm vein in the resting human subject, or from the carotid in the anaesthetized rabbit (ether anaesthesia) or from the severed neck vessels in the fowl has been investigated. The method of collecting the blood has been already described [Conway, 1935] the same procedure being adopted, using a carotid cannula in the rabbit.

Revised method.

Analytical. The method of suspended absorption [Conway, 1935] with the special micro-diffusion "unit" [Conway & Byrne, 1933] has been used, but revised in certain points. The essential differences of the revised method consist first in standardizing the whole procedure with each series of determinations by means of a duplicate control on standard ammonium sulphate solutions. When this is done with the same glassware, all corrections for this and the alkali as well as temperature effects on the absorption rate are eliminated. Also it is recognized that the emission rate of NH_3 from a blood-carbonate mixture is not quite the same as from a water-carbonate mixture. The previous conclusion in this respect [Conway, 1935] was based on a few recoveries under conditions not quite ideal for the comparison, though it was pointed out that a small difference was unimportant. A very large number of such recoveries of comparatively high NH_3 quantities (to avoid appreciable fluctuations of the blood NH_3 throughout the procedure) has since been carried out.

The NH_3 formed by the alkali in addition to the preformed NH_3 in blood has also been carefully studied. It has been found to be strictly linear with the time over a few hours. No essential difference from the previous conclusions has been found, but a temperature factor has been included which, however, for the short absorption periods is scarcely significant. In Table I are incorporated the necessary details for the revised method. The allowances for the alkali action on the blood have been calculated with the help of the formula previously given [Conway, 1935] and correspond with the experimental findings.

Calculation of the blood ammonia using the revised method.

This is shown by an example.

Example 1. (All titrations carried out in duplicate, the control solution containing $2\mu\text{g./ml. NH}_3\text{-N.}$)

Titration of "blank" unit	...	35.6 large divisions
Titration of "analytical" unit	...	31.3 "
Titration of "control" unit	...	14.2 "
Time of absorption	...	10 min.
Room temperature	...	15°

The amount of absorption in the analytical unit corresponds to $35.6 - 31.3$ or to 4.3 , and in the control to 21.4 large divisions (each large division of the burette [Conway, 1934] corresponds to 0.01 ml. of $0.0004 - 0.0005 N \text{ Ba(OH)}_2$).

$$\text{Calculation: } \frac{4.3}{21.4} \times 1.15 \times 2.00 = 0.462 \mu\text{g./ml. NH}_3\text{-N.}$$

The factor 1.15 derives from Table I and 2.00 represents the concentration in the control.

From the gross value of 0.462 , 0.04 is subtracted to allow for the special deaminating action of the carbonate as given in Table I, column 4.

Final result = $0.42 \mu\text{g.}/\text{ml. NH}_3\text{-N}$. Before titrating a series with $\text{Ba}(\text{OH})_2$ it is our custom to run out about 7–10 ml. from the burette, and also to have the cork of the reservoir bottle and of the soda-lime guard painted and sealed with melted paraffin. It will be seen that with the revised method changes in $\text{Ba}(\text{OH})_2$ strength are of no consequence, but it is advisable at the same time to change the solution about once weekly. Table II summarizes a number of “recoveries” of NH_3 from blood, with the error range.

Table I

Absorption time min.	Ratio of NH_3 absorbed from water-carbonate to that from blood-carbonate mixture	The absorption as an approximate percentage of the total NH_3 in the aqueous solution %	Allowance in the final result for the special deaminating action of the alkali at different temperatures, $\mu\text{g.}/100 \text{ ml. NH}_3\text{-N}$				
			25°	20°	15°	10°	0°
10	1.15	52	11	7	4	3	1
12	1.14	57	14	8	5	3	1
14	1.12	61.5	16	10	6	4	2
16	1.11	65.5	19	12	7	5	2
18	1.11	69	22	14	9	5	2
20	1.11	72	25	16	10	6	2

In the above table (made out for the pyrex “unit” as made by Messrs A. Gallenkamp and Co., London) it is understood that 1 ml. blood or water is added to 1 ml. of saturated K_2CO_3 in the outer chamber from pipettes exactly calibrated for this purpose, and that the mixing is carried out with a fixed routine of 15 rotations in approximately 15 sec. The allowances in the fourth column for the special deaminating action of the alkali have a temperature coefficient of 10 % per degree.

Table II. “Recoveries” of NH_3 by the method of suspended absorption (revised)

Fluid examined	NH_3 added per ml. fluid $\mu\text{g. N}$	NH_3 recovered. Mean value $\mu\text{g. N}$	No. of observations	Range $\mu\text{g. N}$
Human blood	91.6	91.8	11	93.6 –88.8
Rabbit plasma	91.6	91.6	6	94.6 –87.1
Water	91.6	91.0	20	95.3 –87.1
Human blood	1.92	1.91	1	—
Human blood	0.98	0.96	5	1.05– 0.88
Human blood	0.39	0.36	2	—

The blood used in the above “recoveries” was freshly shed into an open flask. Usually 10–20 ml. samples were taken, 0.2 ml. of an NH_3 solution in saline being added and a similar amount of saline without NH_3 to a control sample.

For the first five groups 1 ml. of $N/200$ acid with indicator was added to the inner chamber and titrated after the absorption with $N/150 \text{ Ba}(\text{OH})_2$, and for the last three groups the customary solutions for the normal blood NH_3 were used (namely, $N/5000$ acid and $N/2000$ alkali).

Of the 37 results in the first five groups none exceed 4.5 % deviation from the mean value. The coefficient of variation for the group is 2.4 %.

For the most exact procedure possible with the method and the conditions described, the “units” after being filled and ready for the determination, are left aside on the bench for $\frac{1}{2}$ hr., the acid in the central chamber being titrated to the end-point, the fluid removed and replaced by a refill of acid and the lid replaced, after which the determination is carried out. This eliminates any small defect in cleaning and also traces of NH_3 in carbonate and fixative, and with a little experience will be found to give surprisingly good and uniform results.

The method of suspended absorption with plasma and other fluids.

The process of suspending the absorption after a short time makes the micro-diffusion method one of great flexibility in the rapid collection of numerous data, and is advisable when, generally speaking, an error range of 2–5 % for the single determination is not considered too high (the suspension being made after 10–20 min.).

With plasma, the NH_3 comes over at the same rate from a plasma-carbonate mixture as from a water-carbonate mixture so that the factors in column 2 of Table I are not used. The corrections for the extra ammonia in the last column of this table still apply and should be subtracted from the final result.

For all diluted blood and tissue extracts of the order of one in five or more, the calculation is simply the ratio of the NH_3 absorbed to that from the control multiplied by the strength of the control. The absorbed NH_3 is represented as alkali divisions on the burette.

Note on the cleaning of the "units". The following procedure has been found suitable for all purposes. After rinsing with cold and hot water, soap and a test tube brush are used, then the "units" are well rinsed and soaked in dilute acid containing indicator (about $N/200 \text{ H}_2\text{SO}_4$) for 15 min. (the "units" being filled to the top). After repeated rinsing with distilled water they are dried in air.

Results

The normal resting content of NH_3 in human blood.

Using the revised method the previous conclusions [Conway, 1935] were confirmed. The NH_3 content of venous blood from the resting human subject on shedding into an open flask rises swiftly as if from zero to reach a mean value of $0.41 \mu\text{g./ml. NH}_3\text{-N}$ within 5 min., after which it rises at about the rate of $0.006 \mu\text{g./ml. NH}_3\text{-N/min.}$ at 20° . The curve of the median values of successive groups of 11 observations ranged in order of time is given in Fig. 1 (upper curve). The statistical description of variability remains as before [Conway, 1935]. For the time interval of 3–10 min. after open shedding, within which the mean value alters very little, the range found in 28 observations on 12 human subjects was from 0.21 to $0.58 \mu\text{g./ml. NH}_3\text{-N}$, the range being 0.36 – 0.58 when observations on two subjects at the unusually low room temperature of 12° are excluded. Fig. 2 shows the course of the formation in nine subjects for whom a rather extended series was taken. The two lowest of these curves were taken at a room temperature of 12° .

 NH_3 content of rabbit's blood (short ether anaesthesia).

Like the human venous blood there is a rise immediately after shedding into an open vessel. This "alpha" rise begins likewise from a concentration apparently zero—or at least not greater than 1 part in 10 million. The curve of the median values of successive groups of 11 observations on 9 rabbits is given in Fig. 1 (lower curve). (The median values are chosen in order to give the most characteristic curve.) The rabbit curve differs from the human in not reaching an apparent level after the 3rd to the 5th min. and, as will be seen in the next section, a much greater rise in the curve begins between the 10th and 20th min. Values ranging from 0.24 to $0.59 \mu\text{g./ml. NH}_3\text{-N}$ were obtained from the 3rd to the 10th min. (32 observations). Fig. 3 shows graphically the variability and course of the formation for 7 rabbits within the first short period after shedding.

As shown below, the first rapid or "alpha" rise is eliminated by receiving the blood into an atmosphere containing CO_2 .

The NH_3 content of fowl's blood after open shedding.

Three experiments on the fowl's blood showed a similar but much more rapid rise than for the rabbit or human subject. The blood NH_3 appears to rise here also from the zero position.

Fig. 1.

Fig. 2.

Fig. 1. Characteristic curves of the blood ammonia for man (crosses) and rabbit (dots) after shedding into an open flask. Room temperature. Each point is the median of consecutive samples of eleven observations ranged in order of time after shedding.

Fig. 2. Serial curves for nine human subjects, mostly mean of duplicates.

Fig. 3. Serial curves on 7 rabbits, mostly triplicate determinations.

Fig. 4. Serial curves on 3 fowls, means of duplicates.

III. THE NH_3 FORMING IN SHED STERILE BLOOD OF MAN AND RABBIT—ITS STAGES AND ORIGINS

Total NH_3 . The total NH_3 forming in the blood of man and rabbit is shown in Table III. The blood over the long periods was sterilized with thymol or toluene, being stored in vaccine bottles with the usual vaccine technique of capping etc.

It will be seen from Table III that total amounts of approximately $13 \mu\text{g./ml.}$ $\text{NH}_3\text{-N}$ develop in human blood over several days at room temperature and about 20 in rabbit blood.

Table III

Subject	Blood examined	$\mu\text{g./ml. NH}_3\text{-N}$ formed after shedding					
		4 hr.	6 hr.	24 hr.	48 hr.	72 hr.	96 hr.
Rabbit	Whole blood	11.1 (3)	18.8 (3)	18.2 (7)	20.3 (3)	—	24.3 (2)
	Plasma	—	—	3.6 (5)	5.4 (2)	—	—
	Plasma (buffered with borate)	—	—	2.7 (2)	4.0 (2)	—	—
Man	Whole blood	—	—	7.4 (6)	13.1 (2)	13.8 (2)	—
	Plasma	0.7 (2)	—	1.5 (2)	—	—	2.7 (2)
	Plasma (borate buffer)	—	—	1.4 (3)	1.3 (3)	1.3 (1)	—

The numbers in brackets give the totals of individual subjects. All experiments were conducted at room temperature (mean of 18°).

The figure for the whole blood NH_3 in human and rabbit bloods after 24 hr. and longer periods agree with those of Parnas [1925] and of Stanojevic [1938] for the human subject. Earlier figures for the NH_3 in human blood after 24 hr. given by one of us [Conway, 1935] were too low owing to undue exposure of the blood, which then during this time loses NH_3 very appreciably, this exposure being carried out in connexion with a study of the effect of CO_2 loss. All vessels containing blood collected by open shedding throughout the present study were stoppered after about 20 min. initial exposure.

In human plasma 1.3–2.7 $\mu\text{g./ml.}$ develop over 4 days with a mean of 1.4 $\mu\text{g./ml.}$ $\text{NH}_3\text{-N}$. That there is practically no change from 1.4 over 3 days indicates that this may be taken as representing the total amount. The formation in rabbit plasma is greater than this. After 24 hr. about 3 $\mu\text{g./ml.}$ $\text{NH}_3\text{-N}$ have developed at room temperature. The formation has not then ceased but the further increase in a subsequent 24 hr. period does not exceed 1–2 $\mu\text{g./ml.}$

The "alpha" NH_3

This formation is almost complete in the first few minutes after open shedding as shown in Fig. 1.

It amounts to a mean of about 0.4 $\mu\text{g./ml.}$ N for rabbit and man. It does not appear on receiving the blood into CO_2 as already shown for the human subject [Conway, 1935] and here as in Fig. 5 for rabbit blood.

It was previously demonstrated that CO_2 in itself does not produce an apparent absence of NH_3 . This has been repeatedly confirmed both for NH_3 already formed in the blood and for recoveries of minute amounts of added NH_3 . When, however, the blood NH_3 is raised to the high level of 100 $\mu\text{g./ml.}$ N a somewhat reduced recovery—85 %—was found with the revised method allowing the usual 10 min. absorption period. In repeated experiments no such reduced recovery was found at the 1–10 $\mu\text{g./ml.}$ level, even with one atmosphere of CO_2 ; and even if we were to allow this 15 % it would mean a recovery of 0.34 $\mu\text{g./ml.}$ N when 0.40 was really present. Such a difference is not significant for the present discussion.

The sudden lowering of the CO_2 tension in open shedding can act either by raising the pH of the blood or in some specific manner. We have maintained the pH at 7.2 by reception into a maleic acid or a phosphate buffer solution forming about one-tenth of the whole blood collected, and with results shown in Fig. 6 (curve B). It is obvious that this procedure if anything only increases the initial rate of the formation compared with unbuffered blood (curve A) so that we must conclude that CO_2 acts specifically.

It has been shown also [Conway, 1935] that carbamic acid itself is not the substance involved, but that some derivative containing an amino group is liberated, the amino group being then removed by ferment action. Adenosine could possibly fill this role, and the following is some evidence in support of such a view.

Fig. 5.

Fig. 6.

Fig. 5. Blood NH_3 , 2 rabbits. *A* and *B*, open shedding; *C* and *D*, shedding into CO_2 (100 mm.).

Fig. 6. Blood NH_3 , 2 human subjects. *A*, open shedding, unbuffered; *B*, open shedding, buffered (7.2 pH); *C*, shedding into 100 mm. tension of CO_2 .

Evidence that adenosine is the amino substance involved in the "alpha" stage.

(1) Out of 51 different substances investigated of physiological importance and containing amino or volatile amine groups, only adenosine, or substances that can liberate adenosine by dephosphorylation are deaminated in mammalian blood after shedding.

(2) From evidence given subsequently small quantities of adenosine must be continually liberated in plasma.

Fig. 7. Plasma NH_3 formation in 6 rabbits. Room temperature.

(3) The differences in rate of formation of the "alpha" stage in plasma and in whole blood fits in well with the adenosine hypothesis. Small quantities of

adenosine added to whole blood are deaminated in about 1 min. or less, but take upwards of 30 min. in plasma. If the "alpha" rise occurs also in plasma it cannot be faster than this adenosine deamination if adenosine is involved.

Figs. 7 and 8 show the "alpha" rises in rabbit and in human plasma, and as will be seen both take about 30 min. to reach completion.

Fig. 8. Plasma NH_3 formation for 3 human subjects (mean curve) after collecting into CO_2 (100 mm. tension) with subsequent release of the gas by exposure in large flask. The dotted curve shows the NH_3 formation in human whole blood.

The rabbit plasma was obtained by centrifuging immediately after open shedding; the human by centrifuging under 100–200 mm. CO_2 tension and subsequent removal of the CO_2 by shaking a small volume in a large conical flask.

(4) The pH effect on the NH_3 formation in the "alpha" stage has a general correspondence with the effect on the deamination rate of adenosine. The "alpha" rise is inappreciable at 6.0 and 8.0 pH and seems most marked around a pH of 7.0. This question is again considered in the discussion at the end.

The "beta" NH_3 and its phases

The NH_3 deriving from the breakdown of adenylypyrophosphoric acid is here termed the "beta" NH_3 . The NH_3 forming in whole blood after the "alpha" stage is a mixture of this NH_3 and that deriving from another source which is present alone in plasma. This latter is termed the "gamma" NH_3 and is subsequently considered.

The relation of the A.T.P. content to the NH_3 formation

Methods.

(a) *Ba salt determination.* The A.T.P. content was determined by a modification of the method of Parnas & Lutwak-Mann [1935] specially adapted for the absorption unit as used here. In this method the Ba salt is precipitated from a trichloroacetic filtrate and subsequently determined by the action of a muscle extract. This method allows also of a simultaneous determination of adenylic acid in the filtrate after the Ba precipitation but no appreciable quantity was found.

(b) *Determination of the total adenylypyrophosphate, adenylyl diphosphate and muscle adenylic acid.* This is carried out simply by the action of a muscle extract on the laked blood. At first strong extracts were used (1 in 3 to 1 in 5) and the NH_3 largely removed after the addition of sodium carbonate etc. Much weaker extracts, however, may be used directly as was subsequently found (about 1 in

50 of frog or rabbit's skeletal muscle). The extract and laked blood were both buffered with maleic acid buffer (pH of 7.0, $M/20$ in mixture) and mixed in equal volumes for the extract action, suitable controls being set up with extract and blood diluted with similar volumes of water. The total adenylyl NH_3 is formed very quickly after mixing and is complete after about 15 min. at room temperature. The formation was in all cases studied from the time concentration curves.

Results and blood NH_3 comparisons.

The determination of adenylypyrophosphoric acid in whole blood by the Ba salt method gave for 3 rabbits a mean of $9.0 \mu g./ml.$ N and 9.5 with the direct action of the muscle extract. With the Ba salt method no appreciable amount of free adenylic acid was found, and this is in obvious agreement with the results of the second method, which therefore constitutes *an easy and accurate procedure for determining the A.T.P. in blood, and particularly suitable for numerous determinations.*

In 4 hr. at room temperature the total adenylyl (muscle) complex has just disappeared as such from rabbit blood and NH_3 has formed in a practically equivalent relation as shown in Table IV. With human blood it takes about 24 hr.

Table IV

Subject	Total A.T.P. plus adenylic acid, $\mu g./ml.$ amino N	Change in concentration of adenylyl complex $\mu g./ml.$ amino-N	Change in NH_3 $\mu g./ml.$ N	Time after shedding min.
Rabbit	8.4	-5.4	+7.3	180
	9.7	-9.7	+9.1	240
	10.3	-9.8	+10.3	240
Man	8.8	-5.7	+6.2	1440
	10.1	-5.7	+6.1	1440
	A.T.P. (from barium salt)	Change in A.T.P.		
Rabbit	7.8	—	—	—
	11.2	—	—	—
	8.1	-7.1	+(8.8)	225

The bracketed number in last line gives the mean NH_3 -N formation in the 225 min. for many rabbits.

for a 60 % disappearance of the A.T.P. and in this time there is an approximately quantitative appearance of free NH_3 —a slight excess of NH_3 being evident.

A study of the NH_3 formation in the first 4 hr. in rabbit blood is therefore in effect a study of the "beta" NH_3 over the complete range of its formation.

The figures for the total A.T.P. etc. in the two samples of human blood correspond but are somewhat lower than the data assembled by Buell [1935] for the total adenine nucleotide in human blood, passing through the uranium salt, hydrolysis of the nucleotide, removal of the uranium and determination of the free adenine nephelometrically after precipitating the silver salt. She obtained a range of 9.6–14.8 in numerous observations whereas the two samples here gave 8.8 and 10.1. Her determinations would have included both the adenosine-3-phosphoric and the adenosine-5-phosphoric acid groupings, whereas here only the adenosine-5-phosphoric acid grouping (muscle adenylic acid) would be determined.

Stages in the breakdown of adenylypyrophosphoric acid leading to the formation of NH_3

(a) *Carbon dioxide is allowed to escape initially.* It has been recently shown by Kiessling & Meyerhof [1938] that in muscle A.T.P. is not present as such but rather as a dinucleotide yielding A.T.P. and behaving similarly to this in all the reactions hitherto observed. However this may be for the red corpuscle, we shall consider A.T.P. here as the starting point. Seeing that adenyldiphosphate is now considered to be a stage in the breakdown of A.T.P. in muscle [Lohmann, 1935] and that no free pyrophosphoric acid is formed, we may suppose that the A.T.P. is broken down in an analogous fashion in red corpuscles and that the diphosphate is a stage leading to adenylic acid. That this is not the final stage before deamination in shed blood after escape of CO_2 is shown by the following evidence. The deaminase of adenylic acid is known to act very specifically [Schmidt, 1928] and does not act for example on yeast or vegetable adenylic acid. If we compare the actions of laked and diluted blood containing added bicarbonate ($N/10$ in mixture) on solutions of the two nucleotides made up at a $p\text{H}$ of 7.0 we obtain the results shown in Table V. When the nucleotide concentration is very high—1 %—the

Table V

Added nucleotide in the laked blood mixture %	pH	NH ₃ formation $\mu\text{g. N/ml./min.}$ (multiplied by the blood dilution)		Ratio
		Muscle adenylic acid	Yeast adenylic acid	
Rabbit A. Bicarbonate-nucleotide buffering. Blood dilution, 1 in 8.8; 0.05 N bicarbonate in the mixture; neutral nucleotide mixture added to laked blood				
1.0	7.5	12.2	0.890	0.07
0.5	7.7	5.45	0.238	0.04
0.2	7.9	1.32	0.132	0.10
0.1	8.0	0.037	0.032	0.85
0.05	8.2	0.0132	0.0123	0.93
Rabbit B. CO ₂ -bicarbonate-nucleotide buffering. Blood dilution 1 in 5. 0.025 N bicarbonate in the mixture and CO ₂ tension 38 mm.				
1.0	7.4	4.25	0.48	0.11
0.05	7.4	0.43	0.044	0.10

deamination of the muscle adenylic acid goes very rapidly and yeast adenylic acid yields NH_3 at about 12 % of this rate. As the concentration is lowered and the $p\text{H}$ shifts more closely to 8.3 the difference between the deamination rate for the two nucleotides becomes less until at a substrate concentration of 0.05 % nucleotide the NH_3 is formed from both at the same rate. This can be satisfactorily interpreted only by supposing that with low concentrations and a $p\text{H}$ of approximately 8.3 the pure adenylic deaminase is no longer involved, but that the two nucleotides are being dephosphorylated to adenosine, the latter then being deaminated to inosine. (The red corpuscle is rich in adenosine deaminase as shown in a subsequent section.)

Such a conclusion is further supported by the fact that in plasma at a $p\text{H}$ of 8.3 (bicarbonate buffering) the two nucleotides were found to be deaminated at the same rate independently of their concentrations (under other conditions of buffering and reaction the plasma was found to behave very differently).

We may therefore represent the stages leading to the NH_3 formation in shed blood (with escape of CO_2) as follows:

Adenylypyrophosphoric acid $\rightarrow$ adenyldiphosphoric acid + 1 phosphoric acid
 $\rightarrow$ adenylic acid + 2 phosphoric acid $\rightarrow$ adenosine + 3 phosphoric acid $\rightarrow$ inosine + NH_3 + 3 phosphoric acid.

(b) *Blood reaction maintained at pH 7.4 by CO_2 .* The laked blood was maintained at a pH of 7.4 by addition of bicarbonate (approximately $N/40$ in mixture) and a tension of 35 mm. CO_2 . Each tonometer used (about 300 ml. volume) contained 4 ml. mixture, the blood dilution therein being 1 in 5. A comparison of the rates of deamination of vegetable and muscle adenylic acids was made, and at all concentrations the vegetable adenylic was found to be deaminated at only a fraction of the rate (10–12%) of the muscle adenylic acid (as shown in Table V). Clearly the main path of the deamination is here through adenylic acid and not through adenosine.

The "beta" phases. When the course of formation of the "beta" NH_3 is followed in rabbit blood at room temperature it occurs in a series of phases as shown in Fig. 9 (curve A). The same is observed in human blood when maintained at 38° (curve C) and also at room temperature in the fowl's blood (curve B).

Fig. 9. Curves of ammonia formation in shed blood. A, rabbit blood at 18° ; B, fowl's blood at 18° ; C, human blood at 38° ; D, rabbit laked blood (dilution 1 in 2 at 18°).

The curves in Fig. 9 are given as representative and not averaged from the data since this averaging tends to smooth out the inflexions owing to the appearance of these at somewhat different times from one animal to another. For the demonstration it is necessary to make about 30–40 consecutive determinations on the one blood specimen.

The NH_3 formed in each phase is of the order of 2.0–3.0 $\mu\text{g./ml.}$ The reality of these "beta" phases (β_1 , β_2 , etc.) is very demonstrable from the fact that, at low room temperatures, the rise of the second phase may be interrupted for as long as 2 hr., the blood NH_3 remaining constant (as in Fig. 11, curve C). In all

the rabbit blood examined the β_1 phase was specially marked, and so far as we can judge is always easily demonstrable in a consecutive series. It begins in 10–20 min. after shedding and ends about the 50th min.

Origin of the "beta" phases. Possibilities such as sedimentation of corpuscles, phasic corpuscular permeability, the effect of sudden changes of reaction from

Fig. 10. The effects of fluoride and iodoacetate on the NH_3 formation in rabbit's blood. Room temperature. Upper curve (circles), iodoacetate (1 vol. $M/5$ to 20 vol. blood); middle curve (dots), fluoride (1 vol. $M/5$ to 40 vol. blood); lower curve (crosses), control blood (1 vol. of $M/5$ saline to 40 vol. blood).

Fig. 11. Effect of adenylypyrophosphate addition to laked rabbit blood (total blood dilution, 1 in 2.45) pH of mixture, 8.0. A, adenylypyrophosphate addition (maximum $\text{NH}_3\text{-N}$ 69 $\mu\text{g./ml.}$); B, adenylypyrophosphate addition (maximum $\text{NH}_3\text{-N}$ 34.5 $\mu\text{g./ml.}$); C and D, blood controls for A and B. Blood laked and A.T.P. added immediately after shedding. Room temperature 12–13°.

unknown causes were eliminated; the blood being always shaken before an analytical sample was removed, and since the phases occur also in laked blood the question of permeability does not enter. That small changes in reaction are in themselves an immediate cause was excluded by treating the blood with alkaline phosphate ($M/10$ concentration in mixture).

From a consideration of the stages of the breakdown of A.T.P. given above, it might be thought at first that these were related to the phasic deamination. What appears to be immediately involved however is the re-esterification of the formed adenylic acid and possibly adenyldiphosphate, because the action of iodoacetic acid or fluoride converts the "beta" formation into a smooth curve. This is shown in Fig. 10. Small amounts of the reagents were added to the blood so as to make a total $M/200$ solution. The effect is a greater rapidity of NH_3 formation after $\frac{1}{2}$ hr. and a smooth curve. If such reagents are increased to $M/50$, or over, the opposed effect of a diminished rate is obtained. This we may explain by an inhibition of the initial dephosphorylation of the adenylypyrophosphate.

Adenylypyrophosphate addition and the "beta" NH_3

Neutral sodium adenylypyrophosphate, prepared by Lohmann's method [1931] passing through the Ba salt, was added to laked blood producing an increase in the amino-N of $34.5 \mu\text{g./ml. N}$, the blood being 2.45 times diluted. In a second experiment adenylypyrophosphate was added in twice the previous amount. The course of the NH_3 formation was investigated over 4 hr. and the results summarized in Fig. 11. It will be seen that in the first experiment (curves *B* and *D*) the adenylypyrophosphate produced no increase in the NH_3 formation until about 50 min. had elapsed, when the curve rose abruptly. Twice the amount of added substance showed an earlier increase (curves *A* and *C*) and the curve of formation had a slope twice as steep.

With the laked blood without any addition (but diluted to the same extent) the β_2 phase was very long delayed, but then appeared with a well-marked rise. The slow appearance of this phase could be attributed to the unusually low laboratory temperature of $12-13^\circ$.

In the blood therefore and also probably formed after the dephosphorylation in the breakdown of the A.T.P. there is a substance which either inhibits this breakdown or brings about a re-esterification.

As is well known from re-esterification in muscle extracts [Parnas *et al.* 1935], phosphopyruvic acid can bring this about and the prevention of the formation of phosphopyruvic acid by fluoride or iodoacetic acid will lead to a more rapid disappearance of the A.T.P. and increase in the NH_3 formation. This is what we have seen to be the case on adding iodoacetic acid and fluoride ($M/200$) to blood. It would be easier, however, to explain the phasic or cyclical NH_3 formation if a substance actually inhibiting the breakdown of A.T.P. instead of re-esterifying were found. If we were to suppose such a substance effective above a certain concentration and that it was at the same time being continually removed, a brief consideration would show that it could bring about the "beta" phases under discussion.

Table VI

Blood mixture	Total A.T.P. present as $\mu\text{g./ml. amino-N}$ in mixture	$\mu\text{g./ml. NH}_3\text{-N}$ formed in	
		240 min.	1200 min.
I	$69 + 4 = 73$	16.2	32.0
II	$34.5 + 4 = 38.5$	6.4	15.8
Controls for I and II	None added + 4 = 4	1.3	3.1

Fresh rabbit blood was laked and neutral adenylypyrophosphate added to I and II samples, similar volumes of 0.9 % saline being added to control samples. The blood dilution in the mixture was 1 in 2.45.

From the above experiments it appears also that A.T.P. increases the deamination rate in blood in proportion to its concentration in laked blood. Table VI

summarizes the data in this respect. The NH_3 formation for the lowest concentration of A.T.P. in Table VI (blood to which no A.T.P. was added) is somewhat higher than might be expected from the linear relation, but under the conditions this will no doubt include in comparatively appreciable amount the "gamma" NH_3 subsequently discussed.

The pH effect on the "beta" NH_3 formation

This was examined for rabbit blood, M or $M/2$ buffers being added to produce $M/20$ concentrations in the total mixture, and the NH_3 formation examined over several hours. The buffers used were maleic acid, phosphoric acid, CO_2 on the acid side and bicarbonate and carbonate on the alkaline. The pH determinations were carried out either electrometrically on the plasma separated under paraffin or colorimetrically on saline equilibrated across collodion sacs containing blood.

The investigation was carried out from a pH of 7.0 to about 10.5. Fig. 12 summarizes numerous observations, the curves A , B and C representing the 3, 2 and 1 hr. formations. The alkaline optimum lies between 8.5 and 9.0, though at 8.0, 9.1 or 9.2 it is very little less. Beyond 9.5 or 10.0 the NH_3 becomes no doubt appreciably increased by the alkaline action on other sources of NH_3 . This range of the optimum pH agrees with that for the breakdown of adenylypyrophosphate by liver extract, as shown by Barrenscheen & Lang [1932].

The pH effect on the blood NH_3 formation was investigated by Parnas & Heller [1924] who found that it was reduced to a very low figure at a pH of 9.3 and they put the optimum at about 8.1. The difference between such results and those described are due to the use of borate buffers by Parnas & Heller. These have been shown to inhibit dephosphorylation [Hommerburg, 1935] and their effect may be thus indirectly explained in this fashion. The specific quality of the borate buffers in inhibiting the nucleotide has been subsequently recognized and used, e.g. by Heller & Klisiecki [1932].

The pH effect as found in such experiments and summarized in Fig. 12 demonstrates at least that the optimum deamination (on the alkaline side) occurs at the same or approximately the same pH as the alkaline phosphatase optimum—an expected result when we consider the original source as A.T.P. As shown in a subsequent section, the type of buffer is of the greatest importance in studying pure adenylic acid deaminations.

Salt action. Saline alone will inhibit the NH_3 formation when added beyond a molar concentration of 0.1 and at 1.0 M the influence is very marked.

Defibrinated blood and the "beta" NH_3 . Defibrinated rabbit blood shows a very similar NH_3 formation to oxalated blood. It is slightly faster at first and somewhat slower later, but is otherwise the same. The comparison was made on blood from the same rabbit (two experiments).

Fig. 12. The pH effect on rabbit's whole blood at room temperature 18° .

The "gamma" NH_3

This NH_3 derives from a precursor in plasma and corpuscles and its formation is best studied in separated plasma in which no NH_3 derives from A.T.P. or muscle adenylic acid after shedding.

The formation in plasma is very slow. After 24 hr. $1.5 \mu\text{g./ml.}$ are present in human and a little over $3 \mu\text{g./ml.}$ N in rabbit plasma. The figure for human plasma scarcely increases beyond that of the 24 hr. level, as shown when buffered with borate which acts also as a preservative. In rabbit plasma the NH_3 formation is not ended in 24 hr., but goes on at reduced rates as the time advances. The following facts are significant in deciding what the precursor of this NH_3 may be.

(1) *Specificity of deamination in plasma.* Deamination in plasma as in whole blood is extremely specific. As subsequently shown, out of 51 substances examined, of physiological significance and containing an amino or amine group, only adenosine or a few substances yielding adenosine by dephosphorylation are deaminated in mammalian blood after shedding, the CO_2 being allowed to escape.

The substances capable of yielding adenosine by dephosphorylation include the two adenylic acids, and presumably A.T.P.

There are other possible combinations which can yield adenosine, for example, the co-ferment of Warburg, which however is present only in the red corpuscles and only to about 2 % of the total adenylic complex.

It is possible also that the "gamma" NH_3 derives from the adenine deoxy-riboside which in turn may be formed from the corresponding nucleotide.

(2) *Elimination of free muscle adenylic acid or of A.T.P. as possible precursors of the "gamma" NH_3 .* No free muscle adenylic acid or A.T.P. exists in mammalian plasma as shown by the action of voluntary muscle extract. This gives no increased formation of NH_3 , three experiments of the following type being very carefully carried out. In these the blood from a rabbit was centrifuged at once, the plasma removed and the following tubes set up.

- (a) 2 ml. plasma + 0.5 ml. water + 0.1 ml. buffer.
- (b) 2 ml. plasma + 0.5 ml. extract + 0.1 ml. buffer.
- (c) 2 ml. water + 0.5 ml. extract + 0.1 ml. buffer.
- (d) 2.5 ml. water + 0.1 ml. buffer.

The buffer was 0.2 *M* neutral phosphate (6 vol. of 0.2 *M* Na_2HPO_4 and 4 vol. 0.2 *M* KH_2PO_4) and the muscle extract a 1 in 5 extract of voluntary muscle made up 3 hr. previously from another rabbit.

The tubes were left aside for about 1 hr. when the NH_3 content was determined in each by triplicate analyses. The water used was NH_3 -free and subtracting the trace of NH_3 in (d) from that found in the other tubes, we get from (a) the NH_3 in the plasma, from (b) the NH_3 in plasma and extract after the action of the extract, and from (c) the NH_3 in the extract. In exp. 1 of the group, the figures were 0.62, 5.42 and $4.72 \mu\text{g. N/ml.}$ respectively, showing $0.08 \mu\text{g. N/ml.}$ increase due to the extract, a quantity so small that it could arise as an analytical error. The two remaining experiments gave each a zero increase. In one of these the tubes were incubated for 2 hr. at 38° . Under similar conditions small amounts of added adenylic acid were deaminated in a small fraction of the time allotted for the above extract action.

(3) *Elimination of free adenosine as precursor.* Adenosine itself cannot be directly involved since it is deaminated too rapidly, small amounts disappearing in at most a few minutes in whole blood and being also deaminated with some rapidity in plasma.

(4) *Dephosphorylation and the pH effect.* From the foregoing evidence it would appear that likely precursors are vegetable adenylic acid, or alternatively the adenine deoxyribonucleotide. With either of these substances we may suppose a preliminary dephosphorylation with subsequent deamination of the nucleoside. At the pH reached by plasma after shedding with escape of CO₂ vegetable and muscle adenylic acids yield NH₃ at practically the same rate (Table VII) and this can be explained only by a preliminary dephosphorylation.

Table VII

Concentration of nucleotide in mixture	pH	NH ₃ formation in plasma mixture over 24 hr., μ g. N/ml. in		Control plasma mixture
		Muscle adenylic mixture	Yeast adenylic mixture	
Rabbit A. Bicarbonate buffering. Nucleotide mixture added at pH 8.3.				
0.1 bicarbonate in mixtures. Plasma dilution 1 in 2.5. Room temperature				
0.80	8.3	8.92	8.32	1.2
0.16	8.3	9.32	7.94	
0.04	8.3	9.32	9.82	
Rabbit B. Nucleotide buffering. Neutral adenylate added to plasma in equal volumes. Plasma dilution 1 in 2.0				
1.0	7.6 (at end)	35.0	5.8	1.8
Rabbit C. Nucleotide-maleic acid buffering. Maleic acid <i>M</i> /20 in mixture. Plasma dilution 1 in 2.5				
1.0	7.0	72.6	6.3	1.4
0.1	7.0	6.3	2.3

Fig. 13.

Fig. 14.

Fig. 13. The pH effect on the 24 hr. formation of NH₃ in rabbit plasma (lower curve) and the effect of adding vegetable adenylic acid thereto (upper curve). The dotted curve gives the extra NH₃ formed from the adenylic acid.

Fig. 14. The pH effect on the 24 hr. formation of NH₃ in human plasma.

Assuming the substance to be vegetable adenylic acid and the deamination to follow on a preliminary dephosphorylation, then the curve of formation of the

"gamma" NH_3 with respect to the pH of the buffer mixture may be expected to show two peaks, one about pH 8.5–9.0 and the other somewhat to the acid side of neutrality. Such double peaking has in fact been very satisfactorily demonstrated with the peaks at 6.8 and 8.6 respectively (Figs. 13 and 14). Not only did the normal plasma NH_3 appear with a double peaking when plotted against the pH but the extra NH_3 formed after adding vegetable adenylic acid showed an almost identical curve with peaks in the same position (dotted curve in Fig. 13). The buffer mixture used consisted of a phosphate-citrate-borate solution containing equivalent amounts of the three substances and to which small varying amounts of N HCl or of N NaOH were added. To 1 vol. plasma 0.35 vol. of the buffer mixture was added to make approximately an $M/10$ solution with respect to each buffer constituent.

After these experiments a careful examination was made of guanylic acid, cytidylic acid, guanosine and cytidine additions to plasma, for it seemed a possibility that these acids with their derived ribosides might be involved. Cytidine was prepared by the method described by Levene & Bass [1931]. The other substances (British Drug House preparations) were first purified from traces of NH_3 .

Observations were carried out over 24 hr. and at a pH of 6.7—corresponding to the "acid" peak. Guanylic acid, guanosine and cytidine gave negative results. Cytidylic acid formed NH_3 at a similar rate to vegetable adenylic acid. This was due to the fact that commercial cytidylic acid contains vegetable adenylic acid as an impurity. A purer product made in the laboratory gave rise to no NH_3 as found in earlier experiments on whole blood.

(5) *The total organically bound acid-soluble phosphate in plasma and the increase of the free inorganic phosphate.* It is obvious that, if the "gamma" NH_3 derives from vegetable adenylic acid or the adenine deoxyribonucleotide, organically bound "acid-soluble" phosphate must exist in corresponding quantity in plasma. It is estimated (e.g. Kay [1931]) that for dog's plasma this is about $3\text{ }\mu\text{g. P/ml.}$, which corresponds approximately to $1.4\text{ }\mu\text{g. P/ml.}$ or an amount corresponding to the "gamma" ammonia in human plasma. We have carefully determined the acid-soluble organically bound phosphate in three rabbit plasmas, using the spectrophotometric method as described by Bomskov [1932]. Separate curves were used for the free inorganic and the total acid-soluble phosphate, constructed from standard phosphate brought through the whole procedure. The photometric readings were made by an independent observer. In these rabbit experiments (Table VIII) the mean inorganic phosphate was markedly higher

Table VIII

Rabbit	Free inorganic P in plasma $\mu\text{g./ml.}$		Total acid soluble P in plasma $\mu\text{g./ml.}$	
	Immediate	After 24 hr.	Immediate	After 24 hr.
I	69	72	75	76
II	74	77	84	84
III	73	75	78	78
IV	92	97	—	—
V, VI and VII	62	—	—	—

than that in normal human plasma, being $72.0\text{ }\mu\text{g. P/ml.}$ The total acid-soluble phosphate was $79.3\text{ }\mu\text{g. P/ml.}$ The organically bound P, being therefore $7.3\text{ }\mu\text{g./ml.}$, would account for the first 24 hr. formation of $\text{NH}_3\text{-N}$ in rabbit plasma (about $3\text{ }\mu\text{g. N/ml.}$).

It will appear also that if a nucleotide is being dephosphorylated before deamination that the free inorganic phosphate may be expected to show some increase over the 24 hr. period. In each of 4 exp. this increase was found, the mean value being $3.2 \mu\text{g. P/ml.}$ —corresponding to $1.5 \mu\text{g./ml.}$ Though this figure is under the expected amount if all the “gamma” NH_3 proceeds from nucleotide dephosphorylation and subsequent deamination, yet it is quite possible that inorganic phosphate may decrease slightly by ester formation.

(6) *Rate of plasma NH_3 formation and of adenylic acid deamination.* If vegetable adenylic acid is a source of the NH_3 formation in plasma, then the rate of the extra deamination on adding yeast adenylic acid should correspond with the normal formation. As an example of this correspondence the following experiment may be cited. To 2 vol. of plasma 0.7 vol. of the citrate-phosphate-borate-buffer and 0.1 vol. of water were added producing a pH of 8.6 (alkaline optimum). A similar mixture was set up 0.1 vol. of 2 % sodium adenyate. In the first 4 hr. the NH_3 was formed at a mean rate of $0.35 \mu\text{g./ml. N/hr.}$ in the first mixture and at 0.40 in the second. In the next 20 hr. the mean rate in the first fell to $0.14 \mu\text{g./ml. N/hr.}$, but in the nucleotide mixture remained at 0.38. The obvious explanation here is that the concentration of the nucleotide in plasma already present was somewhat above the level at which the substrate concentration ceases to have effect, but as the deamination proceeds a point is reached at which the deamination rate is affected by the concentration and the NH_3 formation falls. In the mixture with added nucleotide it continues unchanged.

(7) *Direct determination of adenine nucleotide in plasma.* If adenine nucleotide exists in plasma then the method of Buell & Perkins [1928] (recently modified by Buell [1935]) could be considered applicable to its determination, whichever modification it may be. In this method the blood nucleotide after deproteinization by trichloroacetic acid is precipitated as the uranium salt, the nucleotide being then hydrolysed to adenine by H_2SO_4 and the adenine determined nephelometrically as the Ag salt—subsequent to the removal of the uranium by NH_3 . The method was applied here to plasma. To 2 vol. of plasma 3 vol. of 13.3 % trichloroacetic acid were added, and the method carried out on each plasma in triplicate, including triplicate blank determinations and duplicate recoveries of added amounts of the vegetable nucleotide (of the same order as the expected content in plasma). The recoveries so far have been rather poor and variable (up to 60 %) but the existence of the nucleotide in plasma appears to be proved, in amounts which can be only provisionally stated as about 5 to 10 mg./100 ml.

Summarizing this evidence, one may say that the “gamma” NH_3 in plasma is formed either altogether or in large part from an adenine nucleotide other than muscle adenylic acid, and that the formation occurs after a preliminary dephosphorylation. Vegetable adenylic acid fulfils the requirements so far considered for the precursor of the “gamma” NH_3 in plasma.

IV. SUBSTANCES DEAMINATED AFTER ADDITION TO SHED BLOOD

A large number of substances containing an amino or substituted amino group was investigated for deamination on adding to shed blood. The object of this investigation was not to study the full possibilities of blood deamination, but rather to make clearer the sources of the blood NH_3 . With this end in view the conditions established were never very far from those in shed blood, the pH range being from 6.7 to 8.3.

Apart from adenosine and high concentrations of muscle adenylic acid (both of which are rapidly deaminated by whole blood) the usual procedure adopted was to introduce into a sterilized vaccine bottle 10 vol. of laked blood (1:1) or of plasma with 8 ml. of thymol water and 3 ml. of neutralized substrate (with or without buffer). A small crystal of thymol was generally added as well. Very occasionally toluene was used instead of thymol. The bottle was capped and sealed with the usual vaccine technique, fluid for analysis being removed through a sterilized hypodermic needle introduced through the cap. The investigations were extended as a rule to 24 hr. or longer at room temperature (mean of 18°). The procedure was occasionally varied with regard to blood volume etc. The whole blood dilution throughout was from 4.2 to 2.5 times, and for plasma from 2.1 to 1.2 times. Two control bottles were always set up, the substrate being omitted from one and the blood from the other, the same volume and other conditions being maintained. The deamination of the substrate in the blood mixture was always reckoned by comparison with these controls.

In the expression of the resulting deaminations where positive results were obtained these are given as $\mu\text{g./ml. N/min.}$ with respect to the original blood.

Negative results were obtained for mammalian blood with the following substances: arginine, alanine, aspartic acid, glycine, glutamic acid, *l*-histidine, ornithine, *l*-tyrosine, valine, proline, *l*-tryptophan, *l*-cystine, cysteine, glutathione, egg-white digest with pancreatic juice (boiled), acetamide, propionamide, asparagine, histamine, adrenaline, tyramine, acetylcholine, choline, colamine, betaine, neurine, glycocylamine, glucosamine, guanidine, ethylguanidine, dimethylguanidine, creatine, creatinine, uric acid, vitamin B₁, allantoin, guanine, adenine, cytosine, guanosine, cytidylic acid, guanylic acid, methylamine, ethylamine, propylamine, butylamine and *iso*amylamine.

Positive results for mammalian blood were obtained with adenosine, adenylic acid (muscle and vegetable), adenylypyrophosphate and thymus nucleic acid (slight action) as shown in Table IX. In fowl's blood guanosine, guanine, adenine, cytidine and cytosine were in addition deaminated rather freely.

In all cases of positive action sufficient time was left for the production of at least $1\mu\text{g./ml. NH}_3\text{-N}$ and usually considerably more. This amounts to about 10 large divisions on the burette using the technique described. It will be seen that the deamination rate of adenosine is very much greater than that of adenylypyrophosphate, vegetable adenylic or muscle adenylic acid when this is at a level of 0.1 % (far exceeding any possible normal value). When the concentration of muscle adenylic acid is increased to as much as 1 % the deamination rate surpasses even that of adenosine. The significance of this is subsequently considered. The order of the adenosine deamination rate is also much greater than that of guanine, adenine etc. in fowl's blood. Guanosine is attacked much more rapidly than adenine, guanine, cytosine and cytidine in fowl's blood, and guanosine deaminase also exists in the fowl's plasma.

Concerning the sources of the substances used in the above investigations, adenylypyrophosphoric acid was prepared by Lohmann's method [1931], muscle adenylic acid by that of Ostern & Parnas [1932], later supplies being received from Messrs Henning, Berlin. Cytidylic acid, cytidine and cytosine were prepared by the methods of Levene & Jacobs [1911], Levene & Bass [1931] and Levene [1903]. Ornithine, betaine and neurine were supplied by Dr Schuchardt, Görlitz, and the remaining substances by the British Drug Houses.

Table IX

Substance	Subject	Blood used	Sub- strate conc. %	$\mu\text{g. N/ml./min.}$ ($\times$ dilution)	pH	Buffer	Temp.
Adenosine	Man	Whole	0.2	1.25 (4)	7.0	Phos.	18
		Plasma	0.2	0.23 (1)	6.7	Maleic	38
	Rabbit	Whole	0.2	5.85 (5)	7.0	Phos.	18
		Plasma	0.3	0.040 (1)	7.0	Maleic	18
	Fowl	Whole	0.03	6.590 (2)	7.0	Phos.	21
		Plasma	0.03	0.007 (1)	7.3	Phos.	25
	Frog	Corpuscles	0.1	6.040 (6)	6.7	Maleic	16
		Plasma	0.1	0.550	6.7	Maleic	16
	Arenicola	Whole	0.03	0.058 (3)	6.7	Maleic	16
Adenylic acid (muscle)	Man	Whole	1.0	3.480 (1)	7.0	Maleic	18
			0.05	0.066 (1)	7.0	Maleic	18
	Rabbit	Whole	1.0	8.900 (2)	7.0	Maleic	18
			0.1	0.020		Maleic	18
		Plasma	1.0	0.126 (1)	7.0	Maleic	18
			0.1	0.011			
			1.0	0.014 (2)	8.3	Bicarb.	20
			0.1	0.014	8.3	Bicarb.	20
	Rabbit	Whole	1.0	0.80 (2)	7.0	Maleic	21
			0.1	—	7.0	Maleic	21
			1.0	0.748 (2)	8.3	Bicarb.	19
			0.1	0.037	8.3	Bicarb.	19
		Plasma	1.0	0.011 (1)	7.0	Maleic	18
			0.1	0.004 (1)	7.0	Maleic	18
Adenylic acid (yeast)	Rabbit	Whole	1.0	0.013 (2)	8.3	Bicarb.	20
			0.1	0.015	8.3	Bicarb.	20
	Rabbit	Whole	0.28	0.169	8.0	No extra	19
			0.15	0.065 (2)	8.0	Buffer	19
			0.015	0.013	8.0		19
Nucleic acid (thymus)	Rabbit	Whole	0.3	0.004 (2)	8.0	No extra	18
			0.3	0.010 (1)	8.0	Phos.	40
			0.3	0.002 (1)	8.0	Phos.	18
Adenine	Fowl	Whole	0.04	0.029 (1)	7.3	Phos.	21
			0.04	0.038 (1)	8.0	No extra	21
Guanine	Fowl	Whole	Sat.	0.060 (1)	7.3	Phos.	21
Cytosine	Fowl	Whole	0.2	0.088 (1)	7.3	Phos.	21
Guanosine	Fowl	Whole	Sat.	0.309 (1)	7.3	Phos.	25
		Plasma	Sat.	0.009 (1)	7.3	Phos.	25
Cytidine	Fowl	Whole	0.1	0.034 (1)	8.0	No extra	21
Adenine- cystosine dinucleotide	Fowl	Whole	0.1	0.014 (1)	7.3	Phos.	25

The bracketed data were obtained from the same blood. The bracketed numbers give the total animals. Buffer strengths ranged from 0.1 to 0.2 *M*.

DISCUSSION

Since small amounts of NH_3 are probably liberated continually into the blood stream it may be more correct to hold that the characteristic value of the blood NH_3 is below the analytical level (about 0.002–0.003 mg./100 ml. $\text{NH}_3\text{-N}$) than that it is zero; though as previously noted [Conway, 1935] the extrapolation of the mean curve formation gives no free NH_3 at zero time.

Among the more reliable results obtained by other observers the nearest to this was found by Markert [1934; 1936] using a slight modification of the

Parnas-Heller method in which he determined the NH_3 spectrophotometrically. Markert placed the NH_3 content in human blood at less than 0.010 mg./100 ml. $\text{NH}_3\text{-N}$ and in 30 experiments found no trace of free NH_3 therein.

For the first or "alpha" rise after shedding—occurring in the first 5 min. and amounting to about 2 % of the total NH_3 that forms—we have given evidence to show it may derive from adenosine. For this, however, it is necessary to suppose that adenosine in minute concentrations is in some way protected by CO_2 from the action of the ferment. This so far has not been demonstrated, though not fully investigated. On the other hand, an undoubted and powerful inhibition by the CO_2 -bicarbonate system has been shown for adenylic acid deaminase, so that the possibility must be considered of minute amounts of free adenylic acid causing this "alpha" rise. The difficulty of explanation here would be one of rate of action. Reckoning from the effect on adenylic acid added to laked blood, about 20 % at most of minute amounts of adenylic acid are deaminated in 5 min. in unchanged blood, and this is derived from a supposition of linearity of action from high substrates downwards. As we have seen the indication is that at the lowest levels the slope of the curve is less. A further factor, however, must also be considered, namely, that the blood immediately after shedding has a temperature not far from 37° and within the first few minutes the deaminating action is no doubt very considerably greater than at 18° . It remains possible, therefore, that the "alpha" NH_3 derives from a minute amount of free adenylic acid.

However this may be, for the "alpha" NH_3 it is certain that the "beta" NH_3 derives from the breakdown of adenylypyrophosphate. In shed blood with an escape of CO_2 dioxide the path has been shown to pass through adenosine, but the deamination is mainly direct under normal conditions. This NH_3 amounts to about 1.0 mg. $\text{NH}_3\text{-N}/100$ ml. both for the rabbit and the human subject, and accounts for 60–75 % of the whole formation. Attention may be drawn here again to the curious phasic appearance of the "beta" NH_3 and its apparent relation to re-esterification processes. The first of these phases is the most characterized and it is only after this phase has ended that added A.T.P. produces extra NH_3 unless added in large quantities.

The "gamma" NH_3 . This is at present considered for plasma alone, though it probably also forms in the red corpuscle. Evidence has been presented that this derives from vegetable adenylic acid or possibly from the deoxyribonucleotide. Assuming the precursor to be vegetable adenylic acid it is the only one of the four adenylyribose compounds, including adenosine, muscle adenylic acid and A.T.P. which can remain for any appreciable time in plasma. Adenosine disappears in the fraction of a minute (it is freely permeable through the red corpuscle) and muscle adenylic acid very probably in the fraction of a second as it passes through a voluntary muscle zone, for voluntary muscle is freely permeable to muscle adenylic acid, and presumably to adenylypyrophosphoric acid.

Consequently as a carrier of adenylyribose grouping to tissues and as a comparatively durable substance with the pharmacological action of its class its significance may be considerable.

SUMMARY

1. A revised method is described for determining the blood NH_3 .
2. The immediate or "alpha" rise of NH_3 after shedding (about 0.04 mg. $\text{N}/100$ ml.) is dependent on the escape of carbon dioxide and not on a $p\text{H}$ change. Evidence is given for its deriving from minute amounts of adenosine or adenylic acid.

3. Following the "alpha" rise in rabbit blood there is a comparatively rapid formation of NH_3 in a series of stages ending in 3–5 hr. at room temperature and deriving from A.T.P. The path is largely through adenosine in shed blood with escape of CO_2 , but mainly from adenylic acid if the CO_2 tension be maintained. This NH_3 in rabbit blood amounts to 50–60 % of the total forming in sterile blood (about 2.0 mg. N/100 ml.). In human blood it amounts to 80 % of this (1.3 mg. N/100 ml.) and forms much more slowly, 50 % only appearing after 24 hr. at room temperature. The optimum pH of its formation is 8.7.

4. An easy method has been described for determining the A.T.P. in shed blood very suitable for serial determinations, and agreeing with the much longer method of Parnas & Lutwak Mann [1935] through the Ba salt.

5. The "beta" phases of which the most marked is the first are abolished by iodoacetic acid or by fluoride (N/200).

6. A.T.P. added to shed rabbit's blood develops NH_3 comparatively rapidly and in proportion to the amount added. NH_3 , however, does not begin to form therefrom (unless large amounts have been added) until after the first "beta" phase.

7. The plasma or "gamma" NH_3 (probably also forming in the red corpuscles) and amounting to about 1.5 and 4 mg. N/100 ml. in rabbit and in human blood respectively, appears to derive largely from yeast or vegetable adenylic acid (or possibly from the deoxyribonucleotide).

8. Of 51 substances of physiological importance containing amino or volatile groups, only the following yielded NH_3 in blood: adenosine, adenylic acid (muscle), adenylic acid (yeast), A.T.P. and thymus nucleic acid—this last giving only minute amounts.

In avian blood adenine, guanine, guanosine, cytosine and cytidine are freely deaminated.

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