The Conversion of Cyanogen Chloride to Cyanide in the Presence of Blood Proteins and Sulphydryl Compounds

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Aldridge & Lovatt Evans (1946) showed that the physiological effects of cyanogen chloride are similar to those of hydrocyanic acid, and demonstrated that cyanogen chloride is converted to cyanide *in vivo*. The mechanism of this conversion has been studied and the results are given in this paper. Most of this work was carried out during the war years and although incomplete, the results are thought to be of sufficient interest to warrant publication now.

METHODS

Cyanide and thiocyanate were estimated in pure aqueous solution or biological media by conversion to cyanogen bromide (CNBr) followed by reaction with pyridine and benzidine (Aldridge, 1944, 1945). Cyanogen chloride (CNCl) was estimated by the same method with the bromination stage omitted. It is therefore possible by this method to estimate CNCl, cyanide and thiocyanate in the same solution: (*a*) reaction with pyridine-benzidine reagent without bromination estimates CNCl; (*b*) reaction with pyridine-benzidine reagent after bromination estimates CNCl (if present) + HCN + HCNS; and (*c*) aeration for 20 min. to remove CNCl + HCN, followed by reaction with pyridine-benzidine reagent after bromination estimates HCNS. Cyanide and thiocyanate were determined in blood, etc., after precipitation of proteins with trichloroacetic acid (Aldridge, 1944). This method can be used for the determination of CNCl provided the tubes are stoppered during centrifuging. CNCl added to the blood-trichloroacetic acid mixture before centrifuging can be recovered quantitatively. It has been found that there is, at low concentrations (up to 10 μg./ml.), no appreciable loss of cyanide from the phosphate buffer, pH 7·4 at 37°, provided the tubes are stoppered. In view of the volatility of CNCl and its lachrymatory properties, weighing has been avoided in the preparation of solutions. An approximate dilution has been made using ice-cold pipettes and the amount actually added to the reaction mixture determined by analysis of an identical dilution in water. If pure CNCl is not available, a solution may be prepared free from cyanide and chlorine by the method of Klemenc & Wagner (1938). In all solutions when cyanide has been estimated, thiocyanate has also been determined after removal of cyanide by aeration; all but a few estimations with serum were negative. The fact that it has been shown that the substance estimated is completely volatilized by 20 min. aeration and also gives the reaction for CNBr after bromination, serves as a constant check that it is cyanide. When estimating cyanide or thiocyanate in the presence of glutathione, the bromination must be carried out in acid solution (trichloroacetic); if glutathione is brominated at neutral pH a substance is produced which gives the same reaction as CNBr with the pyridine-benzidine reagent.

Pure microcrystalline rat haemoglobin was prepared as follows: 60 ml. of defibrinated rat blood was obtained by decapitation of about twelve rats. The cells were washed twice with three times its volume of saline poured into 120 ml. distilled water, mixed and left in the refrigerator overnight. The crystalline haemoglobin was centrifuged and washed with ice-cold water. To remove stroma the oxyhaemoglobin was converted to the more soluble reduced haemoglobin (Dudley & Lovatt Evans, 1921) by suspending in 200 ml. water and equilibrating with a N_2-CO_2 mixture at about 40°. When all the haemoglobin was in solution, the solution was filtered rapidly through a sintered-glass filter with a wad of cotton-wool above the filter, and the filtrate reoxygenated by bubbling air through the solution; the oxyhaemoglobin soon began to crystallize out in microcrystals. After leaving overnight in the refrigerator, centrifuging down and washing with 50 ml. cold distilled water, it was resuspended in 60 ml. saline phosphate buffer (mix equal volumes 0·165 M-NaCl and 0·165 M-NaH_2PO_4 and bring to pH 7·4 with 0·33 M-NaOH using a glass electrode). This preparation is stable for several weeks in the refrigerator and has a total haemoglobin content of 10–14 vol. O_2%. Pure carboxyhaemoglobin was prepared by a method of Wilson (1945) to whom I am grateful for permission to give the details. Methyl nitrite is generated by dropping 30% (v/v) H_2SO_4 into a mixture of equal volumes of saturated $NaNO_2$ and methanol. Wash the gas free of acid spray by bubbling through dilute $NaHCO_3$. Methyl nitrite (10 ml.) is introduced by a glass syringe into a partly evacuated tonometer containing 20 ml. of haemoglobin suspension. After 5 min. equilibration, evacuate slightly, add a further 10 ml. of methyl nitrite and repeat the equilibration. It is necessary to equilibrate rat haemoglobin suspension longer with methyl nitrite than red cells or haemolysates of red cells of other species owing to the insolubility of rat haemoglobin. The excess methyl nitrite is removed by evacuation and equilibration with air six times. This preparation of methaemoglobin has the advantage that unlike that prepared by the $NaNO_2$ and $K_3Fe(CN)_6$ techniques the blood is not contaminated with excess reagents—methyl nitrite is almost insoluble in water and a few evacuations will remove all excess.

Membranes for ultrafiltration were prepared by the method of Wilson & Holliday (1933).

All reactions studied in this paper were carried out either in the isotonic saline-phosphate buffer given above, or Sørensen's phosphate buffer (M/15) at pH 7·4 and 37°.

All spectrophotometric determinations with haemoglobin derivatives were done using the Unicam S.P. 500 Quartz Spectrophotometer.

RESULTS

Cyanogen chloride is fairly stable at neutral pH, hydrolysing slowly to cyanate and chloride. In addition, about 1 % of the cyanogen chloride is converted to cyanide. After purification of cyanogen chloride, by standing over calcium hypochlorite to remove any cyanide present, followed by distillation over calcium chloride and lime, no reduction of the amount of cyanide produced during hydrolysis is found.

Cyanogen chloride reacts rapidly with both serum and red cells (Aldridge & Lovatt Evans, 1946), but cyanide is produced only from the reaction with red cells. As would be expected in view of this, higher percentage conversions of cyanogen chloride to cyanide are obtained with washed red cells than with whole blood. The presence of cyanide was confirmed by the copper benzidine, Prussian blue and thiocyanate reactions in the trichloroacetic acid filtrate after deproteinization and also in the sodium hydroxide used as an absorbent after volatilization of the cyanide from the untreated blood under vacuum. The formation of cyanmethaemoglobin has also been used to confirm the presence of cyanide.

Haemolysis of red cells produced a slight slowing of the reaction rate while dialysis produced a considerable slowing. Dilution of the original blood during dialysis was corrected by bringing all the solutions to the same haemoglobin content which was checked by the acid haematin method.

Table 1. *Conversion of cyanogen chloride to cyanide by various solutions*

(1 ml. CNCl solution was added to 10 ml. of each solution (final concn. CNCl = 32·3 μg./ml.). All solutions were brought to same haemoglobin content, 11·5 vol. O_2%. All solutions except whole blood were in M/15 phosphate buffer pH 7·4 and at 37°.)

	HCN produced (μg.)		
	10 min.	3 hr.	5·75 hr.
Whole blood	26	26	26
Red-cell suspension	36	35	36
Haemolysed red cell	28	31	36
Dialysed red cell	19	25	27

Reduction of the haemoglobin of whole blood and washed red cells by repeated evacuation and equilibration with nitrogen increases the amount of cyanogen chloride converted to cyanide by 15–20 %.

Cyanogen chloride reacts rapidly with rat haemoglobin, no more than 1 % of the original cyanogen chloride added being left after 5 sec. from a mixture of cyanogen chloride (17·3 μg./ml.) and of haemoglobin (5–7 vol. O_2%). When higher concentrations of cyanogen chloride are used, i.e. 50–100 μg./ml., there is an extremely rapid removal of cyanogen chloride in the first 5 sec. followed by a slower reaction. This is probably due to only a small amount of the haemoglobin being in solution. No cyanide is liberated during this initial reaction with haemoglobin. However, if haemolysed red cells (with or without cell stroma) are added to this reaction mixture there is an immediate liberation of cyanide. Addition of plasma or serum produces no cyanide and intact red cells liberate cyanide very slowly from a reaction mixture of cyanogen chloride and haemoglobin. In these experiments cyanide has therefore been produced by a two-stage reaction, the first an extremely rapid one of cyanogen chloride with haemoglobin followed by a slower reaction with some substance present in the red cell and not in the plasma. Oxy-, carboxy- and reduced haemoglobin all react with cyanogen chloride to give a product which releases cyanide on reaction with haemolysed red cells. On the other hand, methaemoglobin reacts with cyanogen chloride, but no cyanide is liberated from the product on reaction with haemolysed red cells. Any cyanide formed would react with the methaemoglobin to form cyanmethaemoglobin; however, cyanide may be estimated in methaemoglobin solutions after the usual trichloroacetic acid deproteinization. Addition of haemolysed red cells to the reaction product of serum and cyanogen chloride does not produce any cyanide. The formation of a compound with cyanogen chloride from which cyanide may be produced is therefore peculiar to haemoglobin among the proteins present in blood.

The addition of the solution obtained by centrifuging red cells after treatment with cyanogen chloride, to methaemoglobin, shows the immediate formation of cyanmethaemoglobin (disappearance of the band at 625 mμ. and formation of a band at 539 mμ.) thus confirming the presence of cyanide. The addition of cyanogen chloride to oxyhaemoglobin and methaemoglobin produces no immediate change of visible spectrum. However, cyanogen chloride appears to react slowly with methaemoglobin to produce cyanmethaemoglobin. After 1 hr. the amount of cyanmethaemoglobin formed in the presence of 35 μg./ml. cyanogen chloride was roughly equivalent to that produced in 5 min. from 0·8 μg./ml. cyanide. It therefore seems probable that the reaction of cyanogen chloride with haemoglobin followed by the liberation of cyanide from this compound does not involve reaction with the haem portion of the haemoglobin molecule. Throughout this paper, therefore, the product of the interaction of cyanogen chloride with haemoglobin has been referred to as CNCl/globin. This has been used instead of CNCl/Hb to avoid confusion with other derivatives of haemoglobin, e.g. cyanmethaemoglobin Met Hb/CN.

An attempt was made to find the essential component for the second stage in the reaction. Sodium fluoride (10^{-2} M) and sodium cyanide (3×10^{-4} M was the maximum we could use for practical reasons) added to haemolysed red cells did not reduce the liberation of cyanide from the CNCl/globin. Heat denaturation of haemolysed red cells yielded an active protein-free filtrate, but the rate of liberation of cyanide was appreciably lowered. An enzyme cannot, therefore, be involved in the reaction. Dialysed red cells gave a definite but slower liberation and a smaller total liberation of cyanide. Ultrafiltrate of haemolysed red cells was active, though if a trace of copper was added and the solution shaken with air, no cyanide was produced. Table 2 shows that in these experiments there was a correlation between the presence of free SH groups and the liberation of cyanide from the CNCl/globin.

Reaction of cyanogen chloride with glutathione

The number of molecules of cyanogen chloride reacting with one molecule of glutathione (Table 3) has been calculated from the titration of —SH groups before and after reaction with cyanogen chloride, by the method of Lucas & King (1932). One molecule of cyanogen chloride reacts with one molecule of glutathione in the initial fast reaction. When the —SH compound is in excess the initial reaction is followed by a liberation of cyanide (Table 4). If, however, all the free —SH groups are removed by reaction with excess cyanogen chloride, the excess cyanogen chloride being removed by aeration, no cyanide is produced even after 4 hr. incubation. Addition of further glutathione causes an immediate liberation of cyanide (Table 5). This indicates that —SH groups are essential for the liberation of cyanide

Table 2. *Correlation between presence of* —SH *groups and liberation of cyanide from* CNCl/*globin compound*

(CNCl/globin compound prepared by adding 1 ml. CNCl solution (284 μg.) to 5 ml. haemoglobin solution (approx. 10 vol. O_2%). After 10 min. 2 ml. of this mixture were added to 2 ml. of the various solutions. Nitroprusside test was carried out on tungstic acid filtrates.)

Reaction mixture	Percentage conversion CNCl→HCN 10 min.	Percentage conversion CNCl→HCN 4 hr.	Nitroprusside test
CNCl/globin + haemolysed red cells } Hb concn. 5·7 vol. O_2%	33	67	+ + + +
CNCl/globin + dialysed red cells } Hb concn. 5·7 vol. O_2%	—	10	+
CNCl/globin + ultrafiltrate of red cells	8	17	+ +
CNCl/globin + ultrafiltrate of red cells + Cu and shaken with air	—	3	—

The second reaction may therefore be mediated through some substance containing SH groups, which is dialysable and is normally present in red cells, but not in plasma. Glutathione added to the CNCl/globin produces cyanide while cysteine, cystine or ergothioneine do not. It would therefore appear that the essential reactions in the production of cyanide from cyanogen chloride and blood are an initial reaction with haemoglobin followed by liberation of cyanide by reaction with glutathione. However, cyanogen chloride reacts directly with glutathione. The rate of the reaction is of the same order as with haemoglobin, the concentration of cyanogen chloride added being reduced to 1% in 5 sec. From the product of this reaction cyanide is liberated at a slow rate, as it is from the CNCl/globin. Since the rate of reaction of cyanogen chloride with haemoglobin and with glutathione is of the same order, it is possible that both reactions contribute to the conversion of cyanogen chloride to cyanide *in vivo*.

The reaction between glutathione and cyanogen chloride has been examined more fully since it also appeared to be a two-stage process similar to that with haemoglobin.

as well as the first stage of the reaction. From titration of —SH groups after the initial reaction and after 4 hr. incubation at 37°, it has been calculated that one molecule of glutathione reacts with the initial reaction product to give one molecule of cyanide (Table 3). It should be noted that in this experiment the —SH groups of the glutathione were protected from atmospheric oxidation during the 4 hr. incubation by the presence of cyanide which forms complexes with traces of catalytic metals.

In view of the above reactions other —SH compounds were examined. Cysteine, the parent —SH amino-acid in glutathione, and *N*-acetylcysteine prepared by the method of Pirie (1931), a substance with an acetyl group in the same position as the glutamyl group on the amino group in glutathione, have been examined. The molecular proportions of the initial reaction with cyanogen chloride were determined by allowing an excess of cyanogen chloride to react with a known amount (5 mg.) of —SH compound, and the amount of cyanogen chloride remaining after the reaction determined. This was done in preference to the determination of —SH groups, in view of the difficulty of obtaining consistent results from the iodine titration of cysteine

Table 3. *Molecules of cyanogen chloride reacting with one molecule glutathione, cysteine or* N-*acetylcysteine*

(1) Reduced glutathione (GSH, 10 mg.) in 5 ml. M/15 phosphate buffer pH 7·4 added to 5 ml. buffer containing an estimated amount of cyanogen chloride. The mixture was incubated at 37° for 10 min. and excess —SH groups were then determined by titration with iodine.

(2) Cysteine (CySH) or N-acetylcysteine (5 mg.) in 5 ml. M/15 phosphate buffer pH 7·4 were added to excess cyanogen chloride in 5 ml. buffer and the mixture incubated at 37° for 10 min. The amount of CNCl remaining was then determined.

The liberation of HCN (slow reaction) was examined by determining further loss of —SH groups by iodine titration and the production of HCN after 4 hr.

(The —SH groups are protected from oxidation during incubation by the presence of HCN.)

	Immediate reaction 10 min., 37°			Slow reaction 4 hr., 37°		
	—SH compound reacting (mg.)	CNCl reacting (mg.)	Molecules CNCl/molecule SH compound	—SH compound reacting (mg.)	HCN produced (mg.)	Molecules SH compound/ molecule HCN
Glutathione	5·52	1·14	1·03	—	—	—
	5·82	1·17	1·0	—	—	—
	4·51	0·952	1·05	2·45	0·226	0·96
	5·45	1·15	1·05	2·68	0·227	1·04
Cysteine	5·0	2·04	1·04	—	—	—
	5·0	1·90	0·98	—	—	—
N-Acetylcysteine	5·0	1·92	1·02	—	—	—

Table 4. *Liberation of* HCN *from product of the reaction of cyanogen chloride with glutathione, cysteine and* N-*acetylcysteine*

(Excess —SH compound in 5 ml. M/15 phosphate buffer pH 7·4 added to 5 ml. CNCl solution (amount estimated given in brackets). HCN was determined at various times.)

Glutathione: 5 ml. GSH solution (5 mg.) +5 ml. CNCl solution (291 μg.)		Cysteine: 5 ml. CySH solution (5 mg.) +5 ml. CNCl solution (810 μg.)		*N*-Acetylcysteine: 5 ml. *N*-acetylcysteine solution (6 mg.) +5 ml. CNCl solution (312 μg.)	
Time (min.)	Conversion CNCl→HCN (%)	Time (min.)	Conversion CNCl→HCN (%)	Time (min.)	Conversion CNCl→HCN (%)
10	12·0	10	4·8	10	4·7
20	20·6	20	4·5	20	6·7
60	43·8	60	4·2	60	9·1
120	64·2	90	4·0	180	17·8
240	62·7	110	To 5 ml. add 5 ml. GSH solution (5 mg.)	200	To 5 ml. add 5 ml. GSH solution (5 mg.)
		120	4·9	210	23·1
		170	4·1	220	25·0
				260	35·5
				320	44·2

Table 5. *Reaction of glutathione and* N-*acetylcysteine with cyanogen chloride*

(—SH compound in 5 ml. phosphate buffer pH 7·4 added to excess CNCl in 5 ml. buffer. Absence of —SH groups was shown by iodine titration after 10 min. incubation at 37°. Excess CNCl was then removed by aeration for 15 min. (absence of CNCl shown by pyridine-benzidine reagent) and the mixture then incubated at 37°. Samples were removed for HCN estimation at various times. Zero time is the time of the original addition of CNCl.)

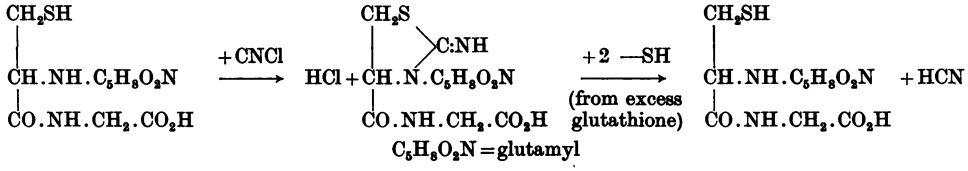
Glutathione: 5 ml. GSH solution (10 mg.) +5 ml. CNCl solution (2·04 mg.)		*N*-Acetylcysteine: 50 ml. *N*-acetylcysteine solution (5 mg.) +5 ml. CNCl solution (2·17 mg.)	
Time (min.)	Conversion CNCl→HCN (%)	Time (min.)	Conversion CNCl→HCN (%)
40	0·8	60	1·3
60	0·8	210	1·3
180	0·9	230	To 5 ml. add 5 ml. GSH (10 mg.)
200	To 5 ml. add 5 ml. GSH (10 mg.)	240	5·5
220	17·1	260	16·5
280	47·4	290	22·5
340	52·8	350	36·5

and *N*-acetylcysteine (Lucas & King, 1932). One molecule of cyanogen chloride reacts with one molecule of both cysteine and *N*-acetylcysteine (Table 3). However, no cyanide is liberated from the reaction product with cysteine even if glutathione is added (Table 4). *N*-Acetylcysteine, on the other hand, liberates cyanide on further incubation. This release of cyanide is rather slow, but is accelerated by the addition of glutathione (Table 4). Like the reaction with glutathione, if all —SH groups are initially removed by reaction with excess cyanogen chloride, no cyanide is liberated until a further supply of —SH groups (glutathione in this case) is provided (Table 5).

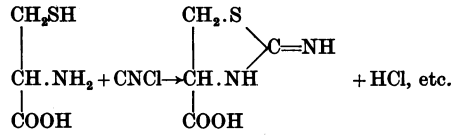
DISCUSSION

In a previous paper (Aldridge & Lovatt Evans, 1946) it was shown that except for the local irritation of the respiratory passages, the physiological response to cyanogen chloride can be explained by its conversion to cyanide in the body. Immediately after the inhalation of cyanogen chloride, cyanide could be detected and determined in the circulating blood, and when cyanogen chloride was added to whole blood *in vitro* there was an immediate appearance of cyanide. The site of this reaction is the red cell, and evidence has been presented that the reaction is a two-stage one, the initial one with haemoglobin giving a product from which cyanide is liberated by glutathione. Subsequently, however, glutathione was shown to react directly with cyanogen chloride to produce cyanide. This reaction also takes place in two stages, the initial reaction being rapid giving a product which liberates cyanide relatively slowly. With intact red cells the production of cyanide from cyanogen chloride is rapid (Aldridge & Lovatt Evans, 1946). This is probably not only due to the high concentrations of haemoglobin and glutathione, but also to the physical conditions therein. We believe, therefore, that both of these mechanisms contribute *in vivo* to the production of cyanide from cyanogen chloride.

From a study of the characteristics of the reaction of glutathione with cyanogen chloride, a reaction similar to that postulated for the reaction of cyanogen chloride with 1:2-dithiols (Aldridge, 1948) can now be suggested:



The evidence for such a reaction is as follows: (*a*) The initial reaction results in the removal of one —SH group per molecule of cyanogen chloride. (*b*) Excess cyanogen chloride, removing all free —SH groups, prevents cyanide liberation and blocks the reaction at stage 2. Addition of glutathione to provide the necessary two atoms of hydrogen results in the immediate liberation of cyanide. (*c*) After the initial reaction one —SH group is removed during the liberation of one molecule of cyanide. According to the above reaction two molecules of glutathione supply two atoms of hydrogen, while one molecule of glutathione is regenerated during the reaction. Since glutathione reacts in this way, cysteine should also react in a similar manner.



However, although it has been shown that one molecule of cyanogen chloride reacts with one molecule of cysteine, no cyanide is produced either in the presence of excess cysteine or when glutathione is added. This difference between the reactions of glutathione and cysteine could be accounted for by suggesting that the ring structure formed from cysteine is more stable than that from glutathione which has the amino group substituted with a glutamyl group. In order to check this point *N*-acetylcysteine has been prepared; this compound should react with cyanogen chloride to produce a structure similar to that with glutathione; this should release cyanide in the presence of excess *N*-acetylcysteine or glutathione. It has been found that this is the case. One molecule of cyanogen chloride reacts with one molecule of *N*-acetylcysteine followed by the liberation of cyanide in the presence of the free —SH groups of *N*-acetylcysteine or of glutathione. If all free —SH groups are removed by reaction with excess cyanogen chloride, no cyanide is liberated until glutathione is added. It therefore seems that vicinal —SH and >NH groups are necessary for the production of cyanide from cyanogen chloride by these reactions.

It has been shown that cyanogen chloride reacts with glutathione, *N*-acetylcysteine, haemoglobin and carboxyhaemoglobin to produce compounds which liberate cyanide on treatment with more glutathione. It is possible that the reaction with haemoglobin is analogous to the initial reactions with glutathione and *N*-acetylcysteine, cyanogen chloride reacting with vicinal —SH and >NH groups

in the globin. Mirsky & Anson (1936) have shown that the available —SH groups in native horse haemoglobin are oxidized by treatment with potassium ferricyanide (i.e. in the conversion of haemoglobin to methaemoglobin). It is probable that treatment with methyl nitrite will also effect a similar oxidation of —SH groups, thus giving an explanation why cyanogen chloride does not react with methaemoglobin to give a product which liberates cyanide on reaction with glutathione. If this hypothesis is correct further work might enable the reaction with cyanogen chloride to produce cyanide to be used as a test for vicinal —SH and >NH groups in enzymes and proteins in general.

It is interesting to note that, in common with the findings of Dixon (1947) that lachrymators react with —SH groups, cyanogen chloride reacts with —SH groups and is also a lachrymator.

SUMMARY

1. Cyanogen chloride reacts rapidly with haemoglobin and with glutathione to produce compounds which on treatment with more glutathione liberate cyanide.

2. It is believed that both reactions contribute to the conversion of cyanogen chloride to cyanide *in vivo*.

3. From a comparison of the reaction of cysteine and the reactions of glutathione and *N*-acetylcysteine with cyanogen chloride a mechanism for cyanide production has been suggested.

4. A possible mechanism of the reaction of haemoglobin with cyanogen chloride is discussed.

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REFERENCES

Aldridge, W. N. (1944). *Analyst*, **69**, 262.
Aldridge, W. N. (1945). *Analyst*, **70**, 474.
Aldridge, W. N. (1948). *Biochem. J.* **42**, 52.
Aldridge, W. N. & Lovatt Evans, C. (1946). *Quart. J. exp. Physiol.* **33**, 241.
Dixon, M. (1947). *Biochem. Soc. Symp.* **2**, 39.
Dudley, H. W. & Lovatt Evans, C. (1921). *Biochem. J.* **15**, 487.
Klemenc, A. & Wagner, G. (1938). *Z. anorg. Chem.* **235**, 427 see *Chem. Abstr.* **32**, 4730.
Lucas, C. C. & King, E. J. (1932). *Biochem. J.* **26**, 2076.
Mirsky, A. E. & Anson, M. L. (1936). *J. gen. Physiol.* **19**, 439.
Pirie, N. W. (1931). *Biochem. J.* **25**, 614.
Wilson, C. & Holliday, E. R. (1933). *Biochem. J.* **27**, 1095.
Wilson, K. M. (1945). Personal communication.

The Effect of the Ingestion of a Hypolipotropic Diet on the Level of Pseudo-Cholinesterase in the Plasma of Male Rats

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In 1943, Faber advanced the hypothesis that the liver serves as the principal locus for the synthesis of plasma cholinesterase (ChE) on the basis of his finding that disturbances of hepatic origin in humans were accompanied by a depression in the activity of plasma towards acetylcholine. This hypothesis has been substantiated by the experimental results of many workers. Brauer & Root (1946) and Ellis, Sanders & Bodansky (1947) found that the plasma of rats receiving carbon tetrachloride, a hepatotoxic agent, showed a decreased ability to hydrolyse acetylcholine. Moreover, the results of Wescoe, Hunt, Riker & Lett (1947), as well as those of Grob, Lilienthal, Harvey & Jones (1947), demonstrated that in human subjects with hepatic insufficiency, the rate of regeneration of plasma cholinesterase following the administration of di*iso*propyl fluorophosphonate was much lower than in subjects with normal hepatic function. Since the cholinesterase activity of human plasma is due predominantly to the activity of pseudo-cholinesterase (Mendel, Mundell & Rudney, 1943), it would therefore seem that the synthesis of pseudo-cholinesterase takes place mainly in the liver and is impaired by hepatic insufficiency.

Accumulation of fat in the liver occurs when the diet is inadequate with respect to its content of lipotropic factors (choline, Best, Hershey & Huntsman, 1932; betaine, Best & Huntsman, 1932; methionine, Tucker & Eckstein, 1937). In animals