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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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 diisopropyl 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

with an average liver fat content of 25–30% (resulting from a hypolipotropic diet), impairment in bromsulphalein elimination and an elevated plasma phosphatase level is to be found in a high proportion of the cases (MacLean, Ridout & Best, 1937; McKibbin, Thayer & Stare, 1944).

Accordingly, it was expected that in animals showing an accumulation of fat in the liver pseudo-cholinesterase activity of the plasma would be lowered. The experiments to be reported here reveal that in male rats this does not occur; there is on the contrary a highly significant elevation in the ChE activity of the plasma brought about by a rise in pseudo-cholinesterase activity, but not in true cholinesterase activity.

METHODS AND MATERIALS

Rats of the Wistar strain were used throughout. They were housed in individual cages provided with a false bottom of coarse wire screen. Three days following their setting out, they were divided into the required number of groups balanced with respect to weight.

The diet was weighed out daily and the following morning the amount left over and the scatter were weighed. From these data the individual daily food consumption was calculated. The rats were 'group-pair fed' on a twice weekly basis.

The composition of the basal diet, inadequate with respect to its lipotropic content, is given in Table 1.

Table 1. *Basal hypolipotropic diet*

Component	%
Extracted peanut meal*	30
Casein	6
Sucrose	39
Beef dripping	15
Salt mixture†	4
Corn oil	5
Vitamin powder‡	1
α -Tocopherol acetate	0.010
C.L.O. concentrate§	0.015

* The peanut meal was exhaustively extracted with dilute ethanol and finally dried with absolute ethanol. The choline content was thereby reduced to between 0.2 and 0.5 mg. choline/g., as determined by microbiological assay.

† See Beveridge & Lucas (1945).

‡ The vitamin powder consisted of aneurin hydrochloride, 500 mg.; riboflavin, 250 mg.; pyridoxine, 200 mg.; calcium pantothenate, 1000 mg.; nicotinic acid, 1000 mg.; folic acid, 50 mg.; *p*-amino benzoic acid, 10 g.; 4-methyl 1:4-naphthoquinone, 100 mg.; and inositol, 50 mg. This was made up to 1000 g. with finely powdered sucrose. Rats eating 10 g. of food/day thus received 50 μ g. of aneurin and proportional amounts of the other vitamins daily.

§ The cod-liver oil concentrate (Ayerst, McKenna & Harrison, Ltd., Montreal) contained 200,000 i.u. vitamin A and 50,000 i.u. vitamin D/g.

The animals were maintained on the diet for 4 weeks, usually, and then sacrificed by exsanguination through the carotid artery under light ether anaesthesia. Blood samples were collected in the presence of a small amount of sodium oxalate, centrifuged immediately and the plasma drawn off for estimating enzymic activity. The livers were immediately

removed, weighed and homogenized in acetone for subsequent estimation of lipids (see Best, Lucas, Patterson & Ridout, 1946). When pseudo-cholinesterase estimations were carried out on the livers, the original homogenate was prepared with water, and samples were taken both for enzymic determinations as well as lipid estimations.

Cholinesterase activity was measured manometrically in 0.025 M-NaHCO₃ as medium saturated with 5% CO₂ in N₂ (pH 7.4). Since rat plasma contains both true cholinesterase and pseudo-cholinesterase (Mundell, 1944), the activity of these enzymes was measured separately, using acetyl- β -methylcholine (Mch) (0.03 M) and benzoylcholine (Bch) (0.006 M), respectively, as substrates, according to the method outlined by Mendel *et al.* (1943). The presence of benzoylcholinesterase (Sawyer, 1945) was excluded by the fact that the activity towards benzoylcholine was susceptible to inhibition by 2.5×10^{-6} M-eserine (see Hawkins & Gunter, 1945).

The activity of other esterases (ali-esterases) which, like pseudo-cholinesterase, can contribute to the hydrolysis of non-choline esters (Easson & Stedman, 1937), was estimated by measuring the rate of hydrolysis of tributyrin (0.008 M) in the presence of 5×10^{-6} M-eserine, a concentration which completely suppresses the activity of pseudo-cholinesterase while leaving that of other esterases towards this substrate unimpaired.

EXPERIMENTAL

(a) *The effect of the ingestion of a hypolipotropic diet on the level of pseudo-cholinesterase in the plasma of adult male rats*

Forty male rats (200–250 g. body weight) were divided into two comparable groups. Group I received the basal hypolipotropic diet, group II the same diet with added choline chloride (0.40%). The experiment was allowed to proceed for 4 weeks, after which time the animals were sacrificed.

Table 2 shows the mean level of activity of pseudo-cholinesterase and true cholinesterase in the plasma of these two groups of rats, together with the average total lipid content of the livers of these animals. It will be seen that the activity of true cholinesterase and of common esterase shows no significant variation under these conditions, whereas that of pseudo-cholinesterase is greatly increased when an inadequate supply of lipotropic factors is provided in the diet. This increase is highly significant, a *P* value of 5.5×10^{-10} being obtained upon analysis of the data.

(b) *Plasma and liver pseudo-cholinesterase levels of adult male rats fed a hypolipotropic diet for various periods of time*

The preceding experiment was repeated using forty-eight animals (200–250 g.) on the basal diet and twenty-four of comparable weight on the supplemented diet. At the end of the second, third and fourth weeks, respectively, sixteen of the former and eight of the latter were killed and blood samples taken for analysis. At the same time estimations

Table 2. *The effect of the ingestion of a hypolipotropic diet and of the same diet supplemented with choline on the activity of the cholinesterases in the plasma of adult male rats*

Group	Diet	Average liver wt. (%)	Average total liver lipids (% wet wt.)	Plasma cholinesterase activity*		Plasma common esterase activity†
				Pseudo-ChE	True ChE	
I	Basal	19.7	30.1	79.8 ± 4.5	58.9 ± 5.1	1850 ± 140
II	Basal + choline chloride (0.4%)	12.2	6.6	36.2 ± 2.7† $t = 6.7$ $P = 5.5 \times 10^{-10}$	59.2 ± 3.0 $t = 0.04$ $P = > 1$ (not significant)	1670 ± 114 $t = 1.02$ $0.3 < P < 0.4$ (not significant)

* Activity is expressed as $\mu\text{l. CO}_2$ evolved in 20 min./ml. plasma.

† The values found in the case of the animals maintained on the choline-supplemented diet are in the same general range as those observed in normal animals (see Mendel, Hawkins & Nishikawara, 1948).

‡ Activity expressed as $\mu\text{l. CO}_2$ evolved from tributyrin (0.008M) in 20 min./ml. plasma. Eserine ($5 \times 10^{-6}\text{M}$) was added to suppress the activity of pseudo-cholinesterase.

Table 3. *The effect of the ingestion of a hypolipotropic diet for various periods of time on the level of liver and plasma pseudo-cholinesterase activity of adult male rats*

Group	Diet	Duration of ingestion (weeks)	Average total liver lipids (% wet wt.)	Liver pseudo-ChE activity (Q_{Bch})*			Plasma pseudo-ChE activity†		
				Mean ± s.e.	t	P	Mean ± s.e.	t	P
I	Basal	2	26.1	2.98 ± 0.20	2.7	0.007	49.4 ± 3.3	4.6	<0.001
II	Basal + choline chloride (0.4%)	2	7.3	2.13 ± 0.19			25.8 ± 1.9		
III	Basal	3	28.13	3.08 ± 0.13	3.1	<0.001	66.2 ± 7.5	3.4	<0.001
IV	Basal + choline chloride (0.4%)	3	6.35	2.31 ± 0.25			27.7 ± 1.6		
V	Basal	4	27.09	3.85 ± 0.23	4.9	<0.001	71.7 ± 6.3	4.3	<0.001
VI	Basal + choline chloride (0.4%)	4	6.50	2.16 ± 0.15			29.5 ± 3.2		

* Q_{Bch} indicates the $\mu\text{l. CO}_2$ evolved/mg. fat-free dry residue of the liver homogenate/hr.

† Activity expressed as $\mu\text{l. CO}_2$ evolved/ml. plasma/20 min.

were made of the pseudo-cholinesterase activities of the livers. For this purpose the livers were rendered as free of blood as possible by perfusion with saline through the left ventricle under pressure. They were immediately removed and homogenized with water, with samples of the homogenates being taken for lipid estimations, determinations of enzymic activity and dry-weight estimations. The pseudo-cholinesterase activity of the homogenate was expressed in terms of $\mu\text{l. CO}_2$ evolved/mg. of the dry fat-free residue/hr. (Q_{Bch}); controls for non-enzymic hydrolysis were run simultaneously.

The results of this experiment are outlined in Table 3. It will be seen from these results that the activity of the pseudo-cholinesterase in the plasma is elevated after 2 weeks' ingestion of a hypolipotropic diet and the level of enzymic activity continues to rise as the period of ingestion of the diet is prolonged. Moreover, the pseudo-cholinesterase activity of the liver (Q_{Bch}) in the animals fed this diet is also above normal, and the level of this enzyme also continues to rise as the period of ingestion of the experimental diet is prolonged.

(c) *The effect of the ingestion of a hypolipotropic diet on the plasma pseudo-cholinesterase activity of weanling male rats*

It has been shown (Griffith & Wade, 1939) that weanling rats maintained on a hypolipotropic diet show an elevated liver fat content within 48 hr. and the amount of liver fat increases for the first 4–6 days, during which time there is no evidence of interference with appetite or growth. It was therefore decided to investigate the plasma pseudo-cholinesterase levels of weanling male rats maintained on a hypolipotropic diet to see if the enzymic activity of the plasma of these animals, which showed liver fat accumulation as did the adult rats in the previous experiments, also showed a corresponding elevation.

Twenty male rats (32–40 g. body weight) were divided into two comparable groups. One half received the hypolipotropic ration, the other the same diet supplemented with choline chloride (0.35%). They were group-pair fed on a daily basis for 6 days, and then killed in the usual manner. Gross examination revealed very fatty livers in the

Table 4. *The effect of the ingestion of a hypolipotropic diet in the level of pseudo-cholinesterase in the plasma of weanling male rats*

Group	Diet	Plasma pseudo-cholinesterase activity*		
		Mean $\pm$ S.E.	t	P
I	Basal	38.4 $\pm$ 3.7	3.1	2.3 $\times 10^{-3}$
II	Basal + choline chloride	25.5 $\pm$ 2.7		

* Activity expressed as μ l. CO₂ evolved/ml. plasma/20 min.Table 5. *The ability of various substitutes to prevent the rise in plasma pseudo-cholinesterase observed in male rats maintained on a hypolipotropic diet*

Group	Diet	Average total liver lipids (% wet wt.)	Plasma pseudo-cholinesterase activity*		
			Mean $\pm$ S.E.	t	P
N-0-1	Basal	31.0	81.3 $\pm$ 8.3	7.0	<0.001
N-1	Basal + methionine (0.5 %)	7.3	28.6 $\pm$ 1.7		
N-0-2	Basal	29.2	96.6 $\pm$ 6.9	7.7	<0.001
N-2	Basal + betaine HCl (1 %)	6.9	38.6 $\pm$ 3.0		
N-0-3	Basal	30.5	90.9 $\pm$ 8.9	2.3	0.037
N-3	Basal + triethyl homologue of choline (0.5 %)	6.3	56.1 $\pm$ 7.4		
N-0-4	Basal	31.8	77.9 $\pm$ 8.5	1.9	7.5 $\times 10^{-2}$ (not significant)
N-4	Basal + cystine (0.4 %)	26.4	102.0 $\pm$ 8.5		
N-0-5	Basal	32.8	78.3 $\pm$ 7.8	0.67	Greater than 0.5 (not significant)
N-5	Basal + fivefold increase in vitamin content of basal diet	27.5	84.2 $\pm$ 4.1		

* Activity expressed as μ l. CO₂ evolved/ml. plasma/20 min.

animals without a choline supplement. Table 4 shows that the plasma pseudo-cholinesterase activity of the animals maintained on this basal diet exceeded that found in the animals ingesting the diet containing the choline supplement and the rise was statistically significant.

(d) *The effect of various supplements on the elevated plasma pseudo-cholinesterase of adult male rats maintained on a hypolipotropic diet*

To determine whether choline is specific in preventing the rise in plasma pseudo-cholinesterase observed in the previous experiments, the effect of various other supplements to the basal diet was investigated. The following five supplements were substituted for a corresponding amount of sucrose in the basal ration: methionine (9.5 %), betaine (1 %), the triethyl homologue of choline (0.5 %), cystine (0.4 %) and a fivefold increase in the vitamin content of the basal ration. It was hoped that the levels of methionine and betaine chosen would possess a lipotropic effect comparable to that of the choline used previously. The inclusion of cystine in the scheme was to determine whether the possible efficacy of methionine in reducing the level of the enzyme to normal was due to its action as a sulphur-containing amino-acid or to its formation of choline by transmethylation (du Vignaud, Chandler, Moyer & Keppel, 1939); to decide this question, cystine was used as a supplement in an amount which would

provide a sulphur content equivalent to that provided by methionine in the experiment in which the latter was used. The triethyl homologue of choline was included because it has been shown by Channon & Smith (1936) to possess lipotropic activity (though somewhat less than that of choline), and by McArthur, Lucas & Best (1947) to be biologically incorporated into the molecule of liver phospholipins. The high vitamin diet aimed at deciding whether some non-specific factor among the vitamins included in the diet was capable of influencing the activity of the enzyme when present at a higher level of intake.

In each experiment twenty male rats (150–180 g.) were used. Half were offered the basal diet, the other half received the same diet, with the supplement under investigation. The schedule was so arranged that each experiment was started 1 week following the initiation of the previous experiment. In each case the diets under consideration were offered for 4 weeks on a group-pair fed basis and the animals were killed in the usual manner.

In Table 5 a summary of the results obtained is presented. It will be seen that methionine, betaine and the triethyl homologue of choline, at the levels provided in their respective experiments, opposed the rise in plasma pseudo-cholinesterase observed when the diet is inadequate in its contents of lipotropic factors. The triethyl homologue of choline was not as efficient in this respect as the other two supple-

Table 6. *The effect of the ingestion of a hypolipotropic diet on the level of pseudo-cholinesterase in the plasma of adult female rats*

Group	Diet	Average total liver lipids (% wet wt.)	Plasma pseudo-cholinesterase activity*	P	
I	Basal	30.41	92.4 ± 9.9	2.74	1.7 × 10 ⁻²
II	Basal + choline chloride	5.49	135.3 ± 12.0†		

* Activity expressed as μ l. CO₂ evolved in 20 min./ml. plasma.

† The level of the enzyme in the plasma of the animals receiving the choline-supplemented diet is higher than that of male rats on a similar diet (see Mundell, 1944).

ments; this may, however, be due to the fact that rats tend to refuse food in which this compound is included, and the level of intake of the substance may not have been optimal. The effect of methionine is not merely that of a sulphur-containing amino-acid because cystine not only fails to reduce the level of plasma pseudo-cholinesterase, but may promote a further elevation, though not in the present experiment a significant one. Its failure to prevent the rise in enzymic activity of the plasma agrees with the finding that cystine cannot replace methionine as a lipotropic agent. A fivefold increase in the vitamin content of the diet had no significant effect on the level of plasma pseudo-cholinesterase.

It would seem, therefore, that the ability of choline, methionine, betaine and the triethyl homologue of choline to prevent a rise in the pseudo-cholinesterase in the plasma of male rats is related to the activity of these compounds as lipotropic agents.

(e) *The effect of the ingestion of a hypolipotropic diet on the level of pseudo-cholinesterase in the plasma of adult female rats*

Twenty female rats (140–170 g.) were divided into two comparable groups. One group received the basal hypolipotropic ration the other, the same ration supplemented with choline chloride (0.35 %). The animals were group-pair fed for 4 weeks and then killed in the usual manner.

From Table 6, which contains the results of this experiment, it can be seen that the level of the pseudo-cholinesterase in female rat plasma does not rise when the intake of lipotropic factors is inadequate; instead, it shows a significant fall.

DISCUSSION

The rise in plasma pseudo-cholinesterase following the ingestion by male rats of a diet containing an inadequate amount of lipotropic agents is unexpected in view of the fact that the liver seems to be the main site of pseudo-cholinesterase synthesis and the

accumulation of fat in that organ has been shown to be accompanied by an impairment of hepatic function. The level of this enzyme in the plasma of female rats shows, however, the expected decrease.

The difference in the response of male and female animals to a deficient intake of lipotropic agents suggests that the hypolipotropic diet is not directly responsible for the elevation observed in the males. Some, at the moment obscure, functional change may be induced in the males.

SUMMARY

1. Ingestion of a hypolipotropic diet leads to a highly significant rise in pseudo-cholinesterase activity in the plasma of adult and weanling male rats. The activity of true cholinesterase and of aliesterases in the plasma of these animals is unchanged.

2. The elevation in plasma pseudo-cholinesterase activity is accompanied by a significant rise in liver pseudo-cholinesterase activity.

3. Elevated levels of plasma pseudo-cholinesterase can be reduced to within the normal range by addition to the basal hypolipotropic diet of the lipotropic agents choline, betaine, methionine and the triethyl homologue of choline.

4. Female rats fail to show this elevated plasma pseudo-cholinesterase level when their diet is hypolipotropic.

5. A deficiency in lipotropic factors does not, therefore, seem to be the direct cause of the elevation in plasma pseudo-cholinesterase observed in male rats. The nature of the mechanism responsible for the elevation remains obscure.

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A Bridge Unit for Filter-paper Chromatography

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In chemical pathology filter-paper chromatography is becoming an analytical tool of increasing importance. In this particular field well established methods now allow for accurate identification of urinary sugars (Partridge, 1948), amino-acids (Dent, 1946) and porphyrins (Sveinsson, Rimington & Barnes, 1949).

In the present communication an apparatus is described that has been tested in this laboratory over the last nine months with satisfactory results. Some modifications in analytical technique are given here by way of example; others are the subject of further research.

Apparatus

The novel feature of this all-glass unit for filter-paper chromatography is the arrangement of the paper strip in the form of a bridge from one flask to another by passing it through a glass sleeve. All parts are provided with standard ground-glass joints, the whole unit being made to the author's specification by Quickfit and Quartz, Stone, Staffordshire.

The unit is easily assembled, and all parts are readily accessible for cleaning. It occupies little space on the laboratory bench, having an overall length of approximately 50 cm. The unit can be accommodated in the incubator or the refrigerator as the analytical procedure may demand, and since it is quite airtight it is highly suited for work with volatile solvents.

Fig. 1 shows the general appearance and mode of assembly of the bridge unit. The two 500 ml. flasks (*D*) form, as it were,

the supporting pillars of the bridge. In practice, therefore, the glass bridge can be made to connect any one pair of flasks and no time need be lost in cleaning out the apparatus when changing from one method of analysis to another.

Parts *A* are T-shaped connecting pieces, which sit with their vertical limb on the flasks, the horizontal limb having one open and one slotted end (slot: 22 × 4 mm.); this latter end connects with part *C* or *B* of the bridge. The drawing of the assembly line of the bridge shows how parts *B* and *C* are arranged. Part *B* is a straight glass tube with cone and socket ends, the cone bearing a 9–10 mm. diameter hole in the ground-glass surface. Part *C* is also a glass tube, but here both ends form sockets and carry holes of the same size which locate with the holes in part *B*. One end of part *C* connects with part *A* to complete the bridge. The filter paper is introduced by opening the unit between parts *B* and *C* passing it either way through the slots into the flasks (Fig. 1), the paper just being held at small points of contact. When wet it sags, but even then it does not make contact with the glass along its broad surface.

The free ends of parts *A* are closed with caps *E* (standard size B34). These wide additional openings (internal diameter 28 mm.) to the unit were incorporated in the design for two reasons: first, they allow for the introduction of solutions and reagents to the unit after the filter-paper strip has been put in position and without disturbing it (a funnel with a semi-S-bent outlet is used for the purpose); secondly, this wide opening allows for the introduction of a pair of crucible tongs with which the filter-paper strip can be guided.

Since the holes in parts *B* and *C* are located in the ground-glass joints, the tubes may be rotated either to close or open them. In the former case ('closed' unit) the apparatus is