## Synthesis of Coenzymes I and II

DURING the past year, two biological methods for the determination of coenzymes I and II in blood have been reported[1,2]. Kohn and Klein, using the method described by Kohn, have reported recently the *in vitro* synthesis of the pyridine coenzymes I and II by human erythrocytes[3]. In their study, "fresh defibrinated blood was incubated in tightly stoppered flasks, or tubes, for 18–24 hours at 29°–35°"; apparently a mixture of red cells, white cells and serum was used. In the presence of added nicotinic acid amide or nicotinic acid, the *in vitro* synthesis of these coenzymes by defibrinated blood had also been observed in this laboratory, but from our experiments it seemed that the red cells stored and carried the enzymes instead of performing the synthesis. Accordingly, we took duplicate samples of venous blood from five normal persons, two patients with myelogenous leukæmia, and one patient with lymphatic leukæmia, and by repeated washing separated the red blood cells, the white blood cells and the serum. In each case, 1 c.c. of venous blood, or its hæmatocrit equivalent of washed cells, was incubated 18 hours with an equal volume of saline which contained 0·25 mgm. of nicotinic acid amide per cubic centimetre. The control tubes contained no nicotinic acid amide and were incubated the same length of time with an equal volume of saline.

Repeated tests show that: (1) the concentration of coenzymes I and II in normal human blood and leukæmic blood is doubled after incubation with nicotinic acid amide; (2) carefully washed erythrocytes, however, from normal blood and leukæmic blood, resuspended in saline or serum, show no increase in the concentration of coenzymes I and II after incubation under similar conditions; (3) the concentration of coenzymes I and II is greatly increased in a suspension of white cells of the lymphoid and myeloid series after incubation with nicotinic acid amide.

The above findings are inconsistent with the statement of Kohn and Klein that the normal erythrocytes accomplish the synthesis of coenzymes I and II after incubation with nicotinic acid, and suggest the tentative hypothesis that nucleated cells are essential for the synthesis of these complex enzymes.

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[2] Vilter, R. W., Vilter, S. P., and Spies, T. D., "Relationship Between Nicotinic Acid and a Codehydrogenase (Cozymase) in Blood of Pellagrins and Normal Persons", *J. Amer. Med. Assoc.*, 112, 420 (Feb. 4, 1939).
[3] Kohn, H. I., and Klein, J. R., "The Synthesis of Cozymase and of Factor V from Nicotinic Acid by the Human Erythrocyte *in vitro* and *in vivo*", *J. Biol. Chem.*, 130, 1 (1939).

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## Benzedrine and Brain Metabolism

THE unquestioned value of benzedrine (phenylisopropylamine) in the treatment of narcolepsy has made it of interest to discover in what ways this drug might influence metabolic events in the nervous system.

It has been known for some time[1] that the presence of amines such as tyramine or isoamylamine brings about a marked diminution in the respiration of brain examined *in vitro*. In spite of the fall, however, of the oxygen uptake of brain tissue respiring at 37° in a glucose phosphate medium, oxidation of the amine[2] may take place with the formation of ammonia and the corresponding aldehyde[3]. The enzyme responsible for the oxidation of the amine has been termed amine oxidase, and it has been proved[3] that certain amines which are but feebly or not attacked by the oxidase nevertheless combine with the enzyme and compete with amines which are vigorously oxidized. Among such amines[4] is benzedrine which, whilst suffering little or no oxidation by the amine oxidase of brain, greatly inhibits the oxidation of tyramine or isoamylamine in the central nervous system.

It has now been found that the fall in brain respiration brought about by the presence of tyramine or isoamylamine can be partially or wholly relieved by the addition to the system of small quantities of benzedrine. This phenomenon is shown by the results given in the following table. The effect of adding benzedrine to brain tissue consuming oxygen in the presence of glucose and of tyramine is greatly to stimulate the oxygen uptake.

OXYGEN UPTAKE AT 37° OF GUINEA PIG BRAIN TISSUE IN PRESENCE OF GLUCOSE-PHOSPHATE AFTER 2 HOURS EXPOSURE TO BENZEDRINE, TYRAMINE AND A MIXTURE OF BENZEDRINE AND TYRAMINE.

| Amine present | $\mu$l $O_2$ uptake in 1 hour | |
|---|---|---|
| | Expt. A. | Expt. B. |
| Benzedrine sulphate (0·03%) | 193·6 | 177·7 |
| Tyramine (0·03%) | 71·1 | 90·1 |
| Tyramine (0·03%) + Benzedrine sulphate (0·03%) | 193·5 | 170·8 |

The facts show that the diminution of brain respiration brought about by the amine $R.CH_2NH_2$ when present at low concentrations is not due, or wholly due, to the presence of the free amine but to the accumulation of the aldehyde R.CHO formed as a result of oxidation of the amine by the amine oxidase. Benzedrine owes its stimulating effect on brain tissue respiration in the presence of $R.CH_2NH_2$ to its inhibitive action on the formation of R.CHO. Owing to the competition between benzedrine and $R.CH_2NH_2$ for amine oxidase, the greater the quantity of the inhibitory amine present the greater is the quantity of benzedrine required to neutralize the inhibition of brain respiration.

The molecule R.CHO, for example, isovaleric aldehyde, has been found to be highly toxic to respiratory processes in brain, and this toxicity is not influenced by the presence of benzedrine.

Benzedrine at low concentrations, for example, 0·001 per cent, has little influence on the oxygen uptake of brain when this respires in a glucose medium in the absence of an inhibitive amine; its stimulating effect is only observable if an amine such as tyramine is also present. At relatively high concentrations benzedrine itself exerts large inhibitive effects on brain respiration. Such effects are due to causes other than aldehyde formation.

Benzedrine does not neutralize the inhibitive action on brain respiration of narcotics such as the barbiturates, or of a drug such as bulbocapnine. Its effect appears to be confined to the amines capable of aldehyde formation in the central nervous system.