of heavy water) with the water of the whole body, independent of the change in the concentration of the dissolved substance. There was no loss of water during the hour by excretion of urine (not counting the urine in the bladder), and the amount of water expired in such a period is negligible. The supposition that a complete distribution of the injected water has occurred, that is, that the concentration of 0·07 per cent, or 0·05 per cent, is the same throughout the whole body, can therefore be controlled by calculating the quantity of water required to give these dilutions, and comparing it with the total body fluid as calculated from the body weight of the animals. The amounts of water required to dilute 6 c.c. of 1·64 per cent heavy water to 0·07 per cent, and 4 c.c. of 1·64 per cent to 0·05 per cent, are 135 c.c. and 127 c.c. respectively. The body weight of each rat was 200 gm.; taking the water content of the body to be 66 per cent of the weight, we calculate that these rats contained 132 gm. of water each. It is, therefore, clear that the water injected, with its heavy water indicator, has distributed itself throughout the entire body in one hour.

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## Linkage of Chemical Changes in Muscle

WE have found recently[1] that addition of phosphoglyceric acid to frog's muscle pulp, poisoned with iodoacetic acid, stops the formation of ammonia which is released by this poison; and we have explained this effect of phosphoglycerate, and a similar action of pyruvic salts in fluoride poisoned muscle, by the hypothesis that the resynthesis of adenosinetriphosphoric acid—which cannot be deaminated directly in muscle—is kept going, in absence of glycogenolysis, by the transport of phosphate groups to adenylic acid from phosphoglyceric acid, or from a related phosphate carrier. This transport we have supposed to be indirect; from the intermediary product of glycogenolysis, the phosphate group being transported to creatine, with formation of phosphocreatine, from which it is transported, in Lohmann's reaction, to adenylic acid as phosphate acceptor. The supposed intermediary reaction of the intermediary phosphorus compound with creatine we have written in our scheme of glycogenolysis[2] as reaction (3).

We have now been able to obtain evidence that phosphocreatine is formed readily in muscle pulp poisoned with iodoacetic acid, when phosphoglyceric acid is present: this phosphate carrier is converted into pyruvic acid. The resynthesis of phosphocreatine out of creatine cannot be produced, in such poisoned muscle, either by free phosphate, or by any intermediary product of glycogenolysis: for example, glyceraldehydphosphoric ester, phosphoglycerol, Harden-Young ester, pyruvic acid, lactic acid, diphosphoglyceric acid. Reaction (3) in our scheme may be written as follows:

$$\underset{\text{phosphoglyceric acid}}{C_3H_5O_4(PO_3H_2)} + \underset{\text{creatine}}{C_4H_9N_3O_2} = \underset{\text{pyruvic acid}}{CH_3CO.COOH} + \underset{\text{phosphocreatine}}{C_4H_8N_3O_2(PO_3H_2)} + H_2O.$$

The intermediary phosphate carrier is probably phosphopyruvic acid, recently discovered in muscle by Lohmann and Meyerhof[3].

As in iodoacetate poisoned muscle pulp phosphoglyceric acid is readily transformed into pyruvic acid, but no pyruvic acid is formed from other intermediary products of glycogenolysis more proximate than phosphoglyceric acid, the point at which iodoacetate interrupts the sequency of glycolysis must be situated above the formation of phosphoglyceric acid, and *not below*, as G. Embden[4] and O. Meyerhof[5] have supposed. Their statement that the oxido-reduction between pyruvic acid and phosphoglycerol does not occur in the presence of iodoacetate, is doubtless correct: but glycogenolysis does not proceed to the formation of phosphoglyceric acid, still less of pyruvic acid.

In the presence simultaneously of pyruvic acid and free phosphates, the formation of ammonia is stopped, and the resynthesis of adenosinetriphosphoric acid is kept going in fluoride poisoned muscle pulp; but when no phosphates have been added—those present in the tissue being converted to esters at the onset of crushing—pyruvic acid has no effect; neither on ammonia formation, which is proceeding rapidly, nor in preventing the splitting of adenosinetriphosphoric acid. A carrier of phosphate groups, transporting these groups to creatine and in this way to adenylic acid is formed, therefore, from pyruvic acid and phosphate—possibly the same as is produced from phosphoglyceric acid.

Pyruvic acid is an intermediary product of anaerobic glycogenolysis, and of lactic acid oxidation in aerobic recovery. As a phosphate carrier specific for the phosphorylation of creatine and adenylic acid can be produced from pyruvic acid and inorganic phosphate, it becomes clear that the same intermediary phosphate carrier may act in the anaerobic and oxibiotic recovery of muscle, leading in both changes to the resynthesis of phosphocreatine and adenosinetriphosphoric acid.

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[1] *Biochem. Z.*, **64**, 272; 1934.
[2] NATURE, **134**, 627, Oct. 20, 1934.
[3] *Biochem. Z.*, **60**, 273; 1934.
[4] Not published, read in Basel, May 1933.
[5] NATURE, **132**, 337 (v.p. 340), Sept. 2, 1933.

## Non-Identity of Vitamin $B_2$ and Flavines

DURING the past two years, we have been working on the isolation of vitamin $B_2$, using the chick for assay work. Only those fractions which had the power of preventing pellagra and allowing normal growth in chicks on the vitamin $B_2$ low ration described by Kline, Keenan, Elvehjem and Hart[1] were considered to be potent in vitamin $B_2$. All our results point to the fact that vitamin $B_2$ and flavines are not identical.

The flavine or the lumiflavine prepared from a liver extract did not protect chicks from pellagra. The animals receiving the flavine showed more severe symptoms of pellagra than those on the basal ration. The liver extract fraction remaining after the flavines had been removed by adsorption on fuller's earth