35. A NOTE ON THE CARDIAC INOSITOL OF THE SHEEP, PIG AND OX

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(Received 4 January 1940)

THE work to be described was undertaken with the object of investigating the nature of the combined inositol shown to be present in the heart muscle of the dog [Winter, 1934]. It was hoped that a more readily available source of heart muscle might be used, and experiments were carried out with sheep's heart, with the surprising result that combined inositol could not be found; nor could any be found in the heart muscle of the pig and ox.

EXPERIMENTAL

In the case of the sheep and pig, hearts were brought in ice from the slaughter house; no such precaution was taken with the ox hearts. Ventricular muscle was always used, minced in an ice-cold mincer, and two 35 g. portions were heated at 100° with 35 ml. of 10% KOH under reflux, one for 30 min. and the other for 5 hr. The work was begun before the publication by Young [1934] of his volumetric method for estimation of inositol in tissues, and it was decided to continue with the method of isolation and weighing used by the writer, with the following modification. The basic Pb precipitate was washed twice with weak NH₄OH (about 0.08*N*), separated by the centrifuge (5 min. at 9000 r.p.m.), suspended in 50 ml. of water, and decomposed with H₂S. The PbS was removed by filtration, well washed with hot water, and the filtrate concentrated to dryness *in vacuo* at 35–40°. About 30 ml. of ether were added to the flask and poured off after standing a for a day at room temperature. 7.5 ml. of water were placed in the flask and, when the solid was dissolved, 90 ml. of strong alcohol. The flask was allowed to stand at room temperature for a day (any longer interval involving risk of loss of inositol), the precipitate was removed by the centrifuge and 25 ml. of water were added to the alcoholic solution, after which the inositol was precipitated as the Ba compound.

In the following Table are given the weights of inositol obtained in each duplicate experiment, expressed as mg. per 100 g. of fresh heart muscle:

		Sheep					
Heated $\frac{1}{2}$ hr.	75.0	39.2	43.6	32.6	48.1	43.9	74.8
Heated 5 hr.	62.6	35.4	56.9	23.7	42.8	63.2	65.8
		Pig					
Heated $\frac{1}{2}$ hr.	31.4	28.6	98.1	60.0			
Heated 5 hr.	30.5	18.6	75.6	38.5			
		Ox					
Heated $\frac{1}{2}$ hr.	74.0	73.2	54.8	72.5			
Heated 5 hr.	51.3	59.0	46.4	71.0			

It will be seen that in the 13 experiments 11 show a decrease of inositol after the longer heating with KOH, and two show an increase. Possible reasons for the latter may be experimental error, or unequal distribution of inositol in

different parts of the heart muscle. The decrease in inositol found in the majority of the experiments suggested that the substance may not be so stable to alkali as is generally believed. In the first paper on the subject [Winter, 1934] the writer accepted the statements in the literature, e.g. Tollens [1914], that inositol, like glycogen, is very resistant to alkali. The matter has been tested by heating 40 mg. inositol in 40 ml. of 5% KOH under reflux at 100° for 5 hr., and comparing the amount recovered with that in parallel experiments in which the alkaline solution was not heated, and was neutralized immediately after addition of the inositol. There was marked destruction of inositol by the alkali; the decrease (average of 4 experiments) was 43.7%. It is not to be expected that the destruction of inositol has been as great in the heart muscle experiments, since the effective strength of alkali would be much reduced by the buffering action of the proteins and the products of their hydrolysis. Some destruction must, however, have taken place, and it is not safe to conclude that combined inositol is absent from the heart muscle of the sheep, pig and ox, since the figures obtained may be the resultant of destruction of free inositol and liberation of some from combination. The complete absence of combined inositol would be surprising in view of the findings with the dog's heart: a reasonable interpretation of the above results is that the combined inositol of the cardiac muscle of the sheep, pig and ox must be much less than that of the dog. It might be argued that since the dog's heart muscle was added to boiling KOH within a minute or two of the death of the animal, while in the case of the slaughter-house material a much longer interval occurred, much of the combined inositol, if present, was broken down by tissue enzymes and free inositol liberated. This is unlikely to be the reason for the failure to demonstrate any appreciable amount of combined inositol in the heart muscle of the sheep, pig and ox, for it was shown that the combined inositol of the dog's heart was similar in amount, whether the tissue was dealt with immediately or was allowed to suffer survival changes for 90 min. at body temperature. The results described above also render it unlikely that the combined inositol of the dog's heart is a substance such as phytin, derived from plant sources in the food and of no metabolic significance; if this were so, it would be expected that more of the substance would be present in the muscle of herbivorous animals such as the sheep and ox, or the omnivorous pig, rather than the carnivorous dog.

SUMMARY

1. It has not been possible to detect combined inositol in the heart muscle of the sheep, pig and ox.
2. Inositol is destroyed to a considerable extent when boiled with 5% aqueous potassium hydroxide.

REFERENCES

- Tollens (1914). *Handbuch der Kohlenhydrate*, p. 664.
Winter (1934). *Biochem. J.* **28**, 6.
Young (1934). *Biochem. J.* **28**, 1435.