CCII. ON THE THERMOSTABILITY OF PROLAN.

By FREDERIC ANDERTON ASKEW
AND ALAN STERLING PARKES¹.

National Institute for Medical Research, London, N.W. 3.

(Received July 21st, 1933.)

MANY authors have confirmed Zondek and Aschheim's [1928; 1929] original experiments on the thermolability of the ovary-stimulating substance found by them in the anterior pituitary lobe and in the urine of pregnant women². All the observations, however, appear to have been made on material heated in aqueous solution, and the possibility that heating the substance in the dry state might give different results has been overlooked. In attempting high vacuum distillation of the active substance from a dried crude extract of urine, we were led to examine the activity of residues subjected to temperatures of 80°, 100° and 150° for an hour in a high vacuum. It has been found that the active substance of urine of pregnancy is not readily destroyed at 100° when heated in the form of a dry powder, although we have confirmed the fact that heating in the presence of water results in complete and rapid destruction.

Methods and material.

Prolan extract. The same urine extract was used throughout the experiments; it was stored at -2° as a dry powder, under which conditions it remains indefinitely stable. The extract was crude; 10 mg. caused ovulation in 39 out of 40, and 5 mg. in 33 out of 65 oestrous rabbits [Parkes and White, 1933]. 10 mg. have therefore been considered as the amount required to cause ovulation with reasonable certainty. The heated samples were tested in the same way, by intravenous injection followed next day by a sterile laparotomy under ether to ascertain if ovulation had occurred.

Heating in dry condition. The samples heated *in vacuo* were spread on the inside of a thin-walled glass vessel, which was then evacuated by a two-stage mercury vapour pump connected through a trap cooled with liquid oxygen. The vacuum attained was higher, probably much higher, than 10⁻³ mm. Hg. The evacuated vessel was heated in a suitable liquid bath.

Samples heated in oxygen or nitrogen were placed in an ampoule, which was then alternately evacuated and filled four times with the required gas dried by passage over P₂O₅. The ampoule was sealed off after the final admission of dry gas.

Heating in aqueous solution. For obtaining an oxygen-free solution, the sample was weighed into an ampoule from which air was displaced by a current of nitrogen. 10 cc. of distilled water, boiled and cooled in a current of nitrogen,

¹ Foulerton Student of the Royal Society.

² To material from this latter source Zondek and Aschheim have given the name "prolan," which is used here for lack of a better one.

were added. During the heating a slow stream of nitrogen was passed through the solution.

In the converse experiments, oxygen was bubbled through the solution during the heating.

Effect of heating in aqueous solution.

Confirmatory experiments on the thermolability of prolan tested in aqueous solution were carried out as shown in Table I. The results show that:

(a) Very brief heating to 100° in water causes destruction. Our actual experimental figures show a loss of not less than 90 % after 5 minutes. Tests on material heated for 1 hour were carried rather further and indicate a loss of not less than 98 %. Destruction is probably complete in both cases.

Table I. *Effect of heating prolan in aqueous solution.*

No. of exp.	Temperature (°)	Duration of heating (hrs.)	Atmosphere	Amount tested (mg.)	Result
3 a	100	1	Oxygen	25	—
1 c	100	1	Nitrogen	30	—
1 d	100	1	Nitrogen	30	—
3 c	100	1	Oxygen	100	—
3 d	100	1	Nitrogen	500	—
6 c	100	$\frac{1}{2}$	Nitrogen	100	—
7 b	100	$\frac{1}{2}$	Nitrogen	250	—
7 a	100	$\frac{1}{2}$	Nitrogen	100	—
9 a	60	1	Nitrogen	10	+
9 b	60	1	Nitrogen	10	—
10 a	60	1	Nitrogen	10	+
10 b	60	1	Nitrogen	10	—
8 a	60	1	Air	20	+
8 b	60	1	Air	20	+
7 c	60	1	Nitrogen	50	—
8 c	60	1	Air	50	+
8 d	60	1	Air	50	+
6 a	60	1	Nitrogen	100	+
8 f	60	1	Air	100	+

(b) The destructive action does not appear to be influenced by the atmosphere in which the solution is heated.

(c) Heating to 60° in water for 1 hour is less destructive. At least 50 % of the activity survives this treatment.

Table II. *Effect of heating prolan in the dry state.*

No. of exp.	Temperature (°)	Duration of heating (hrs.)	Atmosphere	Amount tested (mg.)	Result
GS 7 R	80	$\frac{1}{2}$	Vac.	20	+
GS 9 R	100	1	Vac.	20	+
GS 9 R	100	1	Vac.	10	+
1 a	100	1	Nitrogen	10	+
2 b	100	1	Oxygen	10	+
2 c	100	1	Oxygen	10	—
2 d	100	1	Oxygen	10	+
4 b	100	1	Oxygen	10	+
4 c	100	1	Oxygen	10	+
4 d	100	1	Oxygen	10	+
4 e	100	1	Oxygen	10	+
GS 11 R	100	1	Vac.	5	—
GS 11 R	100	3	Vac.	10	+
GS 12 R	100	3	Vac.	10	+
6 b	150	1	Nitrogen	100	+
8 c	150	1	Nitrogen	50	+
GS 13 R	150	1	Vac.	20	—

Effect of heating in the dry condition.

The results obtained by heating the dry powder were in striking contrast to those given by heating aqueous solutions. The experiments are summarised in Table II which shows that 10 mg. of the dry powder heated to 100° for 1 hour or more gave a positive response in 10 out of 11 experiments. Comparing this response with the known activity of the unheated powder, it is evident that resistance to the treatment must have been almost if not quite complete. Even heating to 150° for 1 hour failed to destroy all activity, five times the usual dose being active after such treatment.

DISCUSSION.

The experiments described above demonstrate conclusively that whereas the ovulation-producing substance of urine of pregnancy is readily destroyed by heat in the presence of water, it is remarkably thermostable in the dry state. This difference in stability to heat confirms the suggestion that the destructive action is hydrolytic in nature [Marshall, 1932] but, unfortunately, it is not easy to see what use can be made of the property in the purification of extracts.

On the other hand, one important practical use can be made of the findings recorded above, *i.e.* in sterilising the extract. Adequate sterilisation for injection has hitherto been a serious difficulty in the administration of urine extracts. Bacterial filters suffer from two drawbacks: (a) the active principle is absorbed on the asbestos unless the p_H is carefully adjusted, and (b) the final product is necessarily a solution, in which form the substance is not stable. By taking advantage of the thermostability in the dry state, it will be easy to enclose within one ampoule the required amount of sterile powder and sterile water, with a suitable arrangement for mixing when required for injection¹.

SUMMARY.

(1) The ovulation-producing substance of urine of pregnancy is readily destroyed by heating in aqueous solution.

(2) The substance is, however, remarkably thermostable in the dry state; exposure to 100° for 1 hour or more causes no detectable loss of activity.

(3) The exclusion of oxygen does not appear to affect the stability either in aqueous solution or the dry state.

¹ Ampoules of this type are already used by pharmaceutical firms for other substances.

REFERENCES.

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