9 hours. The number of these mitoses is relatively constant and cannot possibly account for the increase in the number of Leydig cells.

Line B represents the number of mitoses per thousand epithelial cells in the prostate gland of the same animals. Within the duration of the experiment, the longer the treatment with PU, the greater the number of mitoses occurring in a unit of time, showing that these cell divisions will vary directly with the duration of the hormonal stimulation.

Conclusions. The scarcity of cell divisions among the interstitial cells of the testes of PU-treated animals makes unlikely the conclusion that the increase in the number of these cells is due, except to a slight degree, to their mitosis. Observations were presented which support the opinions of Esaki,[1] and others that the interstitial cells have an extrinsic origin. Other data, to be presented elsewhere, indicate that certain intertubular connective tissue elements are differentiated into active secreting Leydig cells by the action of the gonadotropic hormones of pregnancy urine.

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Relationship Between Inactivated Prolan and Antiprolan.

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Prolan is a protein-containing hormone in which a protein-like component (carrier) is bound to a comparatively small prosthetic group (active part).[1] While dry prolan powder is very stable prolan solutions are easily inactivated by heat, oxidation and ultraviolet rays[1] although neither ultra-red, red or blue light-rays nor roentgen- or radium-rays affect prolan solutions.[2] The thermo- and photosensitivity of prolan must be due to the presence of the protein-like factor bound to the prosthetic group.

The present investigations concern whether the capacity of prolan to form antiprolan is connected with the prosthetic factor, with

[1] Esaki, S., *Z. Mikro. Anat. Forsh.*, 1928, **15**, 368.

[1] Von Euler, H., and Zondek, B., *Skand. Arch. Physiol.*, 1934, **68**, 232.

[2] Zondek, B., *Hormone des Ovariums und des Hypophysenvorderlappens*, 2nd edition, Springer-Vienna, 1935, p. 252.

its gonadotropic effect, or with the physiologically ineffective carrier substance. Twombly[3] succeeded in inducing antiprolan formation with prolan solutions which had been inactivated to as little as 0.3% of their original effectiveness by one hour's heating in boiling water. This and other facts led the author to the conclusion that antiprolan formation could be explained by the formation of protein antibodies. Brandt and Goldhammer[4] achieved similar results. Gonadotropic hormone which had completely or almost completely lost its hormonal activity through boiling, oxidation or ultraviolet radiation still kept its capacity of inducing the formation of antihormonal sera. The authors conclude that the capacity of forming antihormones is linked to the biologically ineffective protein-like carrier present in the hormone.

I. Our experiments had the reverse result. When we inactivated prolan, traces of the active substance always remained. Even after one hour's boiling at pH 5-7 from 0.3 to 1% activity could still be proved. These minute amounts, however, were sufficient to initiate the formation of antiprolan. Rabbits were immunized for 9 weeks, the animals receiving 250 RU of boiled prolan intravenously twice a week and subcutaneously 4 times a week. The prolan solutions were prepared freshly every day, by dissolving dry powder in water. Before injection the solution was boiled for several seconds. In spite of the boiling there remained 2.5 RU of active substance in the 250 RU of prolan which we used. It had, therefore, been inactivated only 99%. After 9 weeks' injections the rabbits had an antiprolan content of 10 PAU* per cc. Similar experiments carried out with active prolan (250 RU per day) resulted in a much higher titer after 9 weeks, *i. e.*, about 90 PAU per cc. These experiments demonstrate that even the most minute amounts of active prolan are able to form antiprolan, the titer, as a matter of fact, being correspondingly lower (about 11%). We achieved quite different results when we used, instead of a fully effective prolan preparation, a preparation derived from the urine of children which was only slightly effective. Ten mg of this preparation, if injected daily brought about antiprolan formation after 9 weeks. The blood titer was 10 PAU per cc. When, however, we repeated the same experiments with prolan from children's urine which had been boiled for

[3] Twombly, G. H., *Endocrinology*, 1936, **20**, 311.

[4] Brandt and Goldhammer, *Klin. Wchenschr.*, 1938.

* 1 PAU = 1 prolan anti-unit is the smallest amount of the antigonadotropic factor required to annihilate the gonadotropic effect of 1 RU prolan in the immature female rat. At least 10 units should be assayed in a test rat.

3 minutes, even 9 months of injection did not suffice to achieve antiprolan formation. Summarizing, therefore, we can say that immunization with prolan totally inactivated by heat cannot initiate antiprolan formation. If inactivation by heat is incomplete the remaining 0.3-1% of effective prolan is sufficient to bring about antiprolan formation.

II. We used the reverse approach in our further experiments. If prolan whose prosthetic group has been destroyed by boiling is said to be able to form antiprolan, prolan which has been inactivated by boiling must be able to bind itself to antiprolan, *i. e.*, to neutralize antiprolan. This, however, is not true, as shown by the following experiments: 100 RU of prolan, heated for one hour at pH 6 was kept in the incubator for 2 hours with 15 PAU of antiprolan.† The 100 RU of boiled prolan could not inactivate the 15 PAU. This was demonstrated by the fact that 10 RU of active prolan which had been added to the mixture (100 RU of boiled prolan + 15 PAU of antiprolan) was inactivated after having been kept in the incubator for 2 hours.

A quantitative experiment is illustrated in Table I.

TABLE I.

Infantile rat	Anti-prolan	Boiled prolan	Active prolan	Gonadotropic reaction	Binding effect of boiled prolan
		A. Control Experiments.			
	mg	RU	RU		
R.3261	—	100	—	—	
R.3262	15	—	12	—	
R.3263	15	—	14	—	
R.3264	15	—	16	—	
R.3265	15	—	18	—	
R.3266	15	—	20	+	
R.3267	15	—	22	+	
		B. Main Experiments.			
R.3255	15	100	12	—	<8 RU
R.3256	15	100	14	—	<6 RU
R.3257	15	100	16	—	<4 RU
R.3258	15	100	18	—	<2 RU
R.3259	15	100	20	+	
R.3260	15	100	22	+	

In the control experiment (R. 3262-3267) the titer of 15 mg antiprolan dry powder was determined. It became apparent that 15 mg of antiprolan was able to inactivate 12-18 RU of prolan (R. 3262-3265) while 20 RU was not inactivated, for R. 3266 showed oestrous reaction (HVR I). Fifteen mg of the antiprolan

† For our experiments we used our antiprolan-acetone-dry powder (Zondek and Sulman, PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 708), which was freshly dissolved in water for every experiment. 1 mg = ca 1 PAU.

which we used contained, therefore, 18 to 19 PAU. A further control experiment revealed that 100 RU of prolan, boiled for one hour at pH 6, no longer brought about the gonadotropic reaction in the infantile rat and had, therefore, been inactivated. (R. 3261.) In the main experiment each time a solution of 15 mg of antiprolan in 1 cc of distilled water, was combined with 100 RU of boiled prolan, dissolved in 2 cc of distilled water, and placed in the incubator for 2 hours. Then increasing quantities of active prolan (12-22 RU), dissolved in 1 cc of water, were added and kept in the incubator for another 2 hours. The total solution (4 cc) now contained 18-19 units of antiprolan, 100 units of prolan inactivated by boiling and 12-22 units respectively of active prolan. The total solution was injected into infantile rats (6 portions in the course of 36 hours) and the gonadotropic reaction was recorded. The experiments showed that in rats Nos. 3255-3258 the vagina had remained closed, that there was no oestrogenic reaction whatsoever and uteri as well as ovaries had remained absolutely infantile. Thus we saw that 18-19 antiprolan units were able to inactivate 12-18 RU of active prolan in spite of the fact that 100 RU of inactivated prolan had been added. Prolan which had been inactivated by boiling, therefore, did not unite with antiprolan in any way whatsoever. One hundred units of boiled prolan could not bind a few units of antiprolan, for this would have been sufficient to produce the prolan reaction.

After 96 hours R. 3259 and R. 3260 showed the gonadotropic reactions (open vagina, oestrous vaginal smear, thick uteri and enlarged ovaries with corpora lutea). In these experiments prolan had been given the opportunity of being effective because there was an excess of it: 20 or 22 RU of prolan were not completely inactivated by 18-19 PAU of antiprolan; some units of active prolan remained and it was those which brought about the gonadotropic reaction.

The above experiments, therefore, demonstrate that prolan inactivated by boiling completely loses its capacity of neutralizing antiprolan.

Conclusion. Boiled prolan is still able to initiate antiprolan formation for one hour's boiling leaves 0.3-1% active substance. These minute amounts of prolan are sufficient for the formation of antiprolan. After complete inactivation through heat prolan is neither able to produce antiprolan *in vivo* nor paralyze antiprolan *in vitro*. Since prolan consists of an active prosthetic group and an inactive carrier (Euler and Zondek '34) we conclude: The capacity of prolan

to initiate the formation of antiprolan through protracted treatment and the capacity of paralyzing antiprolan *in vitro* is immanent in the active prosthetic group and not in the carrier substance which is hormonally ineffective.

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Metabolic Properties of the Regions of the Amphibian Gastrula.

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A number of authors have investigated the oxygen consumption and carbon dioxide production of the amphibian embryo at various stages of its development, with results of considerable interest. But such investigations throw no light upon the properties of the individual regions of the embryo at various stages, although the work of experimental embryologists has furnished us during the last 20 years with fundamental information about these regions and the part they play in the morphogenesis of the organism. In particular, the phase of gastrulation, during which are formed the germ-layers of classical embryology, and which involves the action of the primary organiser in determining the main axial structures of the embryo, merits the closest study.

Only recently have sufficiently delicate chemical methods become available for attacking this problem. Since Rehberg[1] developed the first ultra-micro burette, these methods have been greatly extended by the work of Linderstrøm-Lang, Holter, and their collaborators at Copenhagen. The first application of this kind of technic to the metabolism of the gastrula was made by Heatley,[2] who estimated the amounts of glycogen in the various regions of the gastrula and confirmed by direct chemical analysis the specially marked disappearance of this polysaccharide during the invagination of the roof of the archenteron, through the dorsal lip of the blastopore. All such observations have significance since it is in the dorsal lip of the blastopore and nowhere else during normal development, that the organiser "hormone" is liberated from its inactive combined form.

Wishing to study the metabolic properties of the dorsal lip of the blastopore, as opposed to the ventral ectoderm, where the organiser

1 Rehberg, P. B., *Biochem. J.*, 1925, **19**, 270.
2 Heatley, N. G., *Biochem. J.*, 1935, **29**, 2568.