difference is probably only one of degree. In the mouse, as in the rabbit, a subcutaneous deposit is formed, and in the former animal an abscess is formed at the site of injection. Since the lack of absorption and the manifestations of toxicity are dependent on the physical properties of subtilin in physiological fluids, it seems unlikely that a species difference would be marked.

Such modifications of subtilin as have been tried to date have not greatly modified the physiological absorption or bacteristatic level, even though the products have had somewhat greater *in vitro* antibiotic activity or greatly increased solubility under physiological conditions.

Although the results in this paper indicate that high systemic levels of subtilin are not practical, it is quite possible that subtilin would be beneficial when topical application is indicated. We have observed no reactions which would contraindicate local application.

Conclusions. The antibiotic, subtilin, has been administered to rabbits in a variety of ways, with analyses of blood to determine absorption. The bioassay procedures are described.

Because of precipitation in physiological fluids, injection into subcutaneous tissue, muscle or peritoneal cavity is an ineffective way of reaching bacteristatic levels of subtilin in the blood stream. Administration by mouth or per rectum is likewise unsatisfactory.

A single injection intravenously is accompanied by some danger and gives a blood level of subtilin which is not maintained. A satisfactory level can be maintained, at least for 4 hours, by slow intravenous infusion, without apparent harm to the animal.

A subtilin-pectin complex, which temporarily allows more subtilin to be soluble in physiological saline, a methyl ester of subtilin which is likewise more soluble and which possesses greater antibiotic activity, and a second methyl ester which is much more soluble, are all absorbed in amounts similar to that of unmodified subtilin.

No observations were made which would contraindicate the topical application of subtilin.

Received June 27, 1949. P.S.E.B.M., 1949, **71**.

17306. The Antidiuretic Action of Relaxin-Containing Preparations.*

M. X. ZARROW. (Introduced by Frederick L. Hisaw.)

From the Biological Laboratories, Harvard University, Cambridge, Mass.

The phenomenon of water retention in the female during pregnancy has received a great deal of attention from many investigators. It has been known for some time that both an extract of the posterior pituitary and desoxycorticosterone acetate have antidiuretic action. In addition, Thorn, Nelson, and Thorn[1] have shown that the sex steroids can also produce water retention in the dog. Nevertheless a suitable explanation for the shift in water balance during pregnancy is still lacking. In the present report data are presented to show that extracts from the ovaries of pregnant sows and blood serum of pregnant rabbits, prepared for relaxative activity on the symphysis pubis of the guinea pig, possess an antidiuretic action in the rabbit.

Several different extracts of relaxin were used in these experiments. Preparation J-46 was prepared from unselected ovaries† of the sow according to the method of Albert,

* This investigation was supported in part by a research grant from the Division of Research Grants and Fellowships of the National Institute of Health, U. S. Public Health Service, to Professor Frederick L. Hisaw.

1 Thorn, G. W., Nelson, K. R., and Thorn, D. W., *Endocrinol.*, 1938, **22**, 155.

† Unselected ovaries were obtained from both pregnant and non-pregnant sows.

Money, and Zarrow[2] and showed an activity of 30 guinea pig units (G.P.U.) per mg dry weight in the symphyseal relaxation test (Abramowitz *et al.*).[3] Two extracts, PS-1‡ and OR-24§ were prepared from the ovaries of pregnant sows. Pregnant rabbit serum was obtained from rabbits between the 25th and 28th days of pregnancy, and concentrated with alcohol and acetone (Albert and Money).[4] In addition, control extracts were prepared from beef heart and blood serum of male rabbits. Extensive tests on the relaxin preparation J-46 showed that it contained no estrogenic or progestational activity.

All tests for antidiuretic action were carried out in adult female rabbits of the New Zealand strain weighing from 4.0 to 5.5 kg. The rabbits were placed in a metabolism cage and food and water supplied *ad libitum*. In addition the animals received lettuce thrice weekly. Prior to the test, 24 hour values were obtained for the urine output and water intake for about 2 weeks. Only those rabbits were used that showed a fairly constant output of urine during this period. Injections of the test substances were given subcutaneously 3 times a day for 3 days. The antidiuresis occurred usually within 24 hours after the injections were started and definitely by 48 hours and the response was considered positive if the urine output was decreased by 50% or more and maintained at this level for at least the duration of the treatment.

A sample of the data obtained may be seen in Fig. 1 to 4. It will be noted that the injection of either 10 ml of saline thrice daily for 3 days or a beef heart extract had no effect on the urine output. However, the injection of relaxin (J-46) at a dose level of 1000 G.P.U. 3 times daily decreased the urine output from 180 ml to approximately 27 ml. The same type of response was also obtained with 250 G.P.U. of relaxin injected in the same manner. However, 100 G.P.U. of relaxin gave no effect.

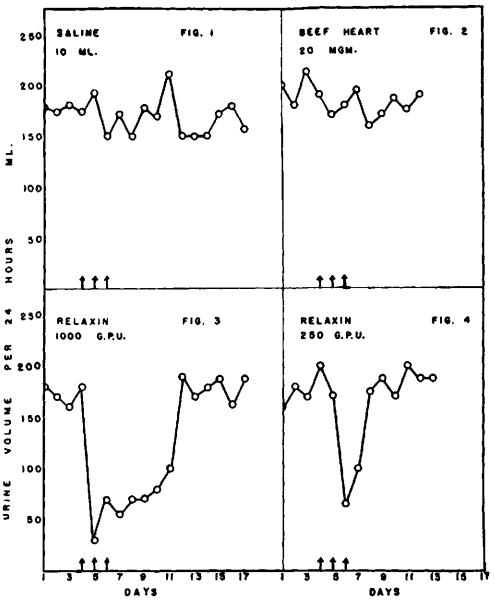

FIG. 1-4.

Twenty-four hour volume of urine in ml plotted against days. Arrows represent days of injection.

FIG. 1. Lack of effect on urine output after treatment with 10 ml of saline injected 3 times daily for 3 days.

FIG. 2. Lack of effect of 20 mg equivalent of beef heart extract injected 3 times daily for 3 days.

FIG. 3. Marked antidiuretic response obtained with 1000 G.P.U. of relaxin (J-46) injected 3 times daily for 3 days.

FIG. 4. Antidiuretic response obtained with 250 G.P.U. of relaxin (J-46) injected 3 times daily for 3 days.

Thus far we have examined a number of preparations for antidiuretic action and have found that relaxin obtained from the ovaries of pregnant sows, nonselected ovaries[3] and pregnant rabbit serum possesses the ability to cause urine retention (Table I). In addition to the controls mentioned above the injection of blood serum from male rabbits also gave negative results. The minimum effective dose of relaxin for the antidiuretic effect was 250 G.P.U. for our preparations and 50 G.P.U. for the Maltine extract. This discrepancy may be explained by the fact that

2 Albert, A., Money, W. L., and Zarrow, M. X., *Endocrinol.*, 1947, **40**, 370.

3 Abramowitz, A. A., Money, W. L., Zarrow, M. X., Talmage, R. V. N., Kleinholz, L. H., and Hisaw, F. L., *Endocrinol.*, 1944, **34**, 103.

‡ Obtained through the courtesy of Dr. Edward H. Frieden, Biological Laboratories, Harvard University.

§ Obtained through the courtesy of Dr. Robert L. Kroc, Maltine Company, Morris Plains, N. J.

4 Albert, A., and Money, W. L., *Endocrinol.*, 1946, **38**, 56.

TABLE I.
Antidiuretic Effect of Relaxin Preparations in the Rabbit.

Relaxin	Dose in G.P.U.*	Source of relaxin	No. of tests	Response
J-46	3000	Ovaries of sows	3	Positive
"	1000	" " "	4	"
"	500	" " "	4	"
"	250	" " "	4	"
"	100	" " "	2	Negative
PS-1	500	Ovaries of pregnant sows	1	Positive
OR-24	1000	Ovaries of pregnant sows	1	Positive
"	500	" " " "	1	"
"	250	" " " "	1	"
"	100	" " " "	1	"
"	50	" " " "	2	"
"	25	" " " "	1	Negative
PR-1	500	Blood of pregnant rabbits	1	Positive
Control	10 ml	Saline	3	Negative
"	1 ml	"	1	"
"	2 ml	"	2	"
"	20 mg	Beef heart	2	"
"	50 ml †	Blood of male rabbits	2	"

* Represents the individual dose that was injected 3 times daily for 3 days.
† The equivalent of 50 ml of blood was injected 3 times daily.

the Maltine unit appears to be approximately 4 times greater than our unit. Thus it would seem that the antidiuretic activity parallels the relaxin activity in extracts prepared in two different laboratories. Furthermore, the presence of the antidiuretic activity in relaxin preparations obtained from such diverse sources as the ovary of the sow and the blood of pregnant rabbits appears to be highly significant. While the evidence is as yet insufficient, there is this striking correlation between the content of relaxin in the preparation and the ability to induce water retention. It may also be pointed out that this new antidiuretic factor is not identical with the posterior pituitary hormone. Donaldson[5] has shown that the latter is dialyzable whereas relaxin and the antidiuretic factor are non-dialyzable.[3] The probability that relaxin is responsible for water retention is also supported by the fact that the blood serum of pregnant rabbits produces both relaxation of the symphysis pubis and water retention whereas the blood serum of male rabbits is without these effects.

The possible identity of the antidiuretic action with relaxin and the fact that the latter substance is found primarily in high concentrations during pregnancy,[6-8] leads to speculation as to whether relaxin may be concerned with the shift in water balance during gestation.

Summary. Relaxin containing extracts of the ovaries of pregnant sows and of the blood of pregnant rabbits possess an antidiuretic action in the rabbit. Some evidence is presented to indicate that the antidiuretic activity parallels the relaxin activity of the preparations used in the present study.

[5] Donaldson, W., *J. Clin. Invest.*, 1947, **26**, 1023.

[6] Marder, S. N., and Money, W. L., *Endocrinol.*, 1944, **34**, 115.

[7] Zarrow, M. X., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 488.

[8] Hisaw, F. L., and Zarrow, M. X., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 395.

Received July 5, 1949. P.S.E.B.M., 1949, **71**.