Table III. Analysis of variance of all irradiated groups except two groups (days $8^1/_2$ and 9)

	Sums of squares	Degrees of freedom	Mean square	Variance ratio	P
Between groups	4292.83	6	715.47	1.413	>0.05
Within group	185267.09	366	506.19		
Total	189559.92	372			

Table IV. Analysis of variance of the control group

	Sums of squares	Degrees of freedom	Mean square	Variance ratio	P
Between groups	4411.57	8	551.446	1.860	>0.05
Within group	20413.39	693	296.412		
Total	209824.96	701			

Table V

7			$8^1/_2$		
Embryos	Number of cells	Number of mitoses	Embryos	Number of cells	Number of mitoses
I	505	48	I	15309	1294
II	434	61	II	7032	903
III	469	50	III	4975	531
IV	1069	102	IV	9960	1105
V	700	96	V	6656	1041
VI	664	119	VI	7265	983
Total	3841	476	Total	51197	5857
$\chi^2 = 34.12$		d.f. $=5$	$\chi^2 = 299.75$		d.f. $= 5$
		$\chi^2 = 3.177$	d.f. $= 1$		

ference in the incidence of resorption and of weight is clear-cut between these two days.

We counted all the cells and mitoses on every second section. The number of cells and mitoses are presented in Table V.

The embryos of the same day show a great variability as far as the number of cells and mitoses are concerned, the difference within a day being significant. On the contrary, no significant difference in mitotic activity could be established between the two days of gestation mentioned.

CORLISS[4], who studied the mitotic activity in the rat embryo on $8^1/_4$, $8^1/_2$, and $8^3/_4$ days, did not find any greater activity of the primitive streak. On the other hand, he found a rise in mitotic activity on the $8^3/_4$ day, but since he attempted no statistical analysis of that fact, no final conclusion can be drawn.

As far as the percentage of gross malformation is concerned, there is no significant difference[1] between the $8^1/_2$ and $9^1/_2$ days, although the mean foetal weights are different.

Thus, after analysing the foetal weight following x-ray irradiation, our conclusion is the same as that made after analysing the incidence of resorption. The irradiation damage, measured as foetal weight, is greater during the mesoderm formation than before or after it.

In view of the results obtained so far, it is reasonable to exclude greater mitotic activity as the main reason for the increased radiosensitivity observed.

Résumé. Les embryons de rat ont été irradiés par les rayons X à la dose de 100 r du cinquième au dixième jour de la gestation. Tous les embryons ont été fixés au bouin le quinzième jour de la gestation et ont été pesés après le passage dans l'alcool.

Les embryons irradiés, comme c'est déjà connu, sont plus légers que les témoins, mais il y a aussi une différence significative entre les divers groupes irradiés.

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Pineal Body and Urinary Sodium Excretion in the Rat

FARRELL et al., in a series of papers[1–5], suggested that the pineal body or some adjacent structure secretes a hormone (adrenoglomerulotropin) which controls the secretion of aldosterone. Although their results were corroborated by other authors[6–10], the role played by the pineal body in the control of the aldosterone secretion has not been definitely settled[11–13]. Until now, only extracts prepared from beef and human pineal body have been investigated either for their aldosterone-stimulating activity or sodium-retaining effect.

In the present experiments, rat's pineal body extracts were used because this animal presents the advantage of having its pineal body far from other brain structures, such as the subcommissural organ. This anatomical feature permits the removal of the gland completely free from the neighbouring tissue.

In this communication we investigated the urinary excretion of sodium as an indication of the aldosterone-stimulating activity of extracts from rat pineal body. The extracts were prepared as follows: the glands were re-

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moved and put into a vial maintained in a salt-iced mixture. The time elapsed between the death of the animals and the cooling of the tissue did not exceed 3 min. The pineal bodies were then homogenized with a tissue grinder in cold saline. The homogenate was centrifuged at 2000 r.p.m. at −4°C, for 15 min. The supernatant was collected and tested for its sodium-retaining activity in a group of 18 rats. Parallel experiments were run with an extract of cerebral cortex (neopallium) similarly prepared and tested in a group of 12 rats. Each animal of these groups received 1 ml supernatant corresponding to 8 mg of fresh tissues, either from pineal body or cerebral cortex, respectively.

In a third group of 18 rats, 10 μg aldosterone[14] were injected; and finally a control group (18 rats) received 1 ml saline. All substances were given by intramuscular injections. Immediately after the injections each rat was put into a metabolic cage and 3 urine samples (A, B and C) were collected at 3, 6 and 9 h respectively.

The animals were allowed to eat and drink *ad libitum* until 3 h before the experiments. The urinary sodium was determined by flame photometry. The results of our experiments summarized in Table I show that the differences between the values of sodium excretion in the groups injected with pineal body extract and aldosterone were not significant. On the other hand, these values when compared with those obtained in the control groups (cerebral cortex extract and saline) were lower for the samples A and B and the differences were statistically significant. These data show that the rats injected with pineal body extract presented a strong sodium retention comparable to that obtained by 10 μg of aldosterone. Sodium retention caused by pineal extract persisted for at least 9 h.

In order to verify whether this sodium retention was mediated through the adrenal gland, parallel experiments were carried out in rats two days after adrenalectomy. Two groups of 11 adrenalectomized rats were injected with pineal body and cerebral cortex extract respectively. Table II shows no significant difference between the sodium excreted by the adrenalectomized rats injected with pineal body and with cerebral cortex extract. When a comparison is made between the results obtained in Tables I and II, it becomes evident that pineal body extract does not cause sodium retention in the adrenalectomized animals. This difference between the intact and adrenalectomized rats strongly suggests that the sodium retention observed in the former, after injection of the pineal body extract, was due to some active substance liberated from the adrenal gland.

These results are consistent with FARRELL's standpoint concerning the existence in the pineal body of a factor (adrenoglomerulotropin) which stimulates the secretion of aldosterone. It has been suggested[4,5] that, despite the fact that the adrenoglomerulotropin might be found in the pineal body, this does not mean that it really is produced there. Similarly, as to what occurs with ADH in the hypothalamo-hypophyseal system, adrenoglomerulotropin could be merely stored in the pineal body after being elaborated by some other mesencephalic or diencephalic areas. Anatomical findings suggest that this does not happen, at least in the rat. The connections of the pineal body in this animal have recently been studied by KAPPERS[15]. According to him, neither nervous nor vascular relations could be observed, pointing to the existence of an epithalamo-epiphyseal or habenulo-epiphyseal complex comparable with the hypotalamo-hypophyseal system. Thus we are led to assume that the sodium-retaining effect produced by our extracts of the rat pineal body is dependent on a factor, probably adrenoglomerulotropin, actually elaborated in the gland[16].

Table I. Urinary sodium excretion of rats injected with pineal body and cerebral cortex extracts, aldosterone and saline

Groups[b]	Urinary sodium excretion (μEq)[a]		
	Sample A (1–3 h)	Sample B (3–6 h)	Sample C (5–9 h)
Pineal body extract (I)	32.4 ± 5.9	36.2 ± 7.1	35.2 ± 7.0
Cerebral Cortex extract (II)	129.5 ± 28.9	81.9 ± 10.2	54.4 ± 9.9
Aldosterone (III)	29.4 ± 8.0	26.7 ± 8.1	41.0 ± 8.9
Saline (IV)	102.4 ± 12.4	68.1 ± 9.4	38.8 ± 5.5

[a] Figures represent mean ± S.E.
[b] 't' test between groups I and III, $P > 0.05$ for samples A, B and C; between groups I and II, $P < 0.01$ for samples A and B, > 0.5 for sample C; between groups I and IV, $P < 0.01$ for sample A, < 0.05 for sample B, > 0.05 for sample C.

Table II. Urinary sodium excretion of adrenalectomized rats injected with pineal body and cerebral cortex extract

Groups[b]	Urinary sodium excretion (μEq)[a]	
	Sample A (1–3 h)	Sample B (3–6 h)
Pineal body extract (I)	86.5 ± 15.9	110.3 ± 15.2
Cerebral cortex extract (II)	111.4 ± 19.5	106.6 ± 19.0

[a] Figures represent mean ± S.E.
[b] 't' test between groups I and II, $P > 0.05$ for samples A and B.

Zusammenfassung. Ein salinischer Extrakt des *Corpus pineale* erzeugt bei normalen, nicht aber bei adrenalektomierten Ratten eine starke Retention von Natrium. Auf Grund der besonderen anatomischen Verhältnisse des Corpus pineale der Ratte vertreten wir die Auffassung, dass die wirksame Substanz, wahrscheinlich Adrenoglomerulotropin, in der Zirbeldrüse selbst erzeugt wird und nicht aus der Nachbarschaft dort angereichert wird.

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[14] Aldosterone was extracted with 70% ethanol from a ground pill (D-aldosterone acetate, CIBA) and saline was added to make a volume five times greater. For the kind gift of the aldosterone pills, we wish to thank Dr. F. UBATUBA from the Institute Oswaldo Cruz, Guanabara (Brazil).

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