zyme; the complex boundary migrates with mobility intermediate between those of considered proteins. As shown in the diagrams of Figure 2 the formation of two relatively stable complexes depends upon the mixing ratio of the proteins.

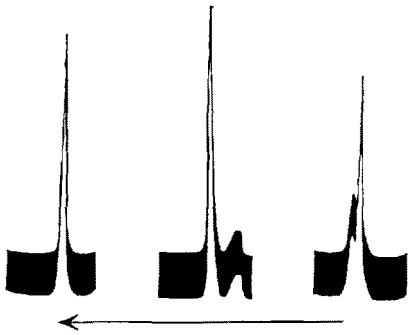

Fig. 2.—Electrophoretic patterns of pepsin-lysozyme complex. From left to right: mixture of pepsin and lysozyme in equal proportions (weight); mixture of pepsin and lysozyme with a little excess of lysozyme; mixture of pepsin and lysozyme with a little excess of pepsin. Total protein concentration: 1·2%. Miller & Golder buffer, pH 8·0, ionic strength 0·1. The photographs are taken from the ascending limb; arrow indicates the anodic migration.

From the calculations based on pH-mobility curve the isoelectric point of the complex falls at pH 7·3.

Electrophoretic examinations conducted at pH above 10 revealed that protein complex persists also on the alkaline side of the isoelectric point of the cationic protein.

Measurement of sedimentation constant in the Phywe Ultracentrifuge gave values of $S_{20} = 4{\cdot}9 \times 10^{-13}$ for the pepsin-lysozyme complex, of $S_{20} = 3{\cdot}1 \times 10^{-13}$ for pepsin and of $S_{20} = 1{\cdot}6 \times 10^{-13}$ for lysozyme.

The results of the present investigation seem of interest in that they demonstrate the ability of pepsin to bind lysozyme, and they may reconcile differences in degrees of interpretation of gastric juice electrophoretic patterns reported by various workers.

A. Caputo

Istituto Regina Elena per lo studio el la cura dei tumori, Rome, November 20, 1954.

Riassunto

L'autore ha studiato il complesso che si forma dall'interazione della pepsina con il lisozima.

Vengono riferiti i risultati ottenuti studiando le caratteristiche torbidimetriche, elettroforetiche e di sedimentazione all'ultracentrifuga del complesso pepsina-lisozima.

Il complesso ha il punto isoelettrico a pH 7·3, ed ha una costante di sedimentazione $S_{20} = 4{\cdot}9 \times 10^{-13}$.

The Effects of 9 α-Halohydrocortisones and of Aldosterone on Survival, Growth and Sodium-Potassium Excretion in Adrenalectomized Rats

The impetus given to biological research on the adrenal cortex by the discovery and isolation of aldosterone[1] and the synthesis[1] of 9-α-chloro- and 9-α-fluoro-hydrocortisone acetates (Cl-F and F-F respectively) needs no emphasis. In the present work the activity of these steriods has been assessed by different methods.

Experiment No. 1: Survival. Male and female hooded x white crossbred rats (initial mean weight 80 g) were arranged in groups of 8 and all were bilaterally adrenalectomised. Physiological saline was given immediately as postoperative drinking fluid. Next day saline was replaced by tap water, as the only source of fluid intake during the experiment, and daily injections of the respective steroids were commenced. The rats were fed the ordinary pellet diet[2] usual in the Department. Drinking fluid and food were given *ad libitum*. Daily injections of steroids dissolved in 20% ethanol in water were administered subcutaneously.

One group of 8 rats was used as control and received only injections of the solvent. The experiment was planned to last 28 days. Spontaneous long survival of a small percentage of adrenalectomized rats is a common feature and for this reason the *median survival time* (M.S.T.) has been recorded, i.e. the period (days) elapsed until 50% of the rats in each group died. This criterion is justified[3] since the number of deaths in adrenalectomized rats, as shown by preliminary experiments, follows a normal distribution.

The M.S.T. in the *control* group was 8 days. Rats which received 0·75 μg *aldosterone*, on the other hand, were all alive after 8 days. At this stage administration had to be discontinued owing to shortage and solvent only was substituted. The M.S.T. of this group was 13 days. This result was submitted to statistical analysis and the difference between the two median survival times proved to be highly significant.

Administration of either Cl–F or F–F at a minimal dose of 10 μg or more kept all rats alive. These results support the work of Borman, Singer, and Numerof[4] although a strict comparison of results is not possible since these workers administered only a *single* injection of an aqueous suspension of the steroids, while in this experiment *daily* doses of aqueous-alcoholic solution were given.

At the end of this experiment several rats were used for a long-term survival and pregnancy experiment. The results on pregnancy are reported elsewhere[5]. In the long-term survival experiment it was found that indefinite survival could be obtained with daily injections of either 10 μg of Cl–F or 50 μg of F–F.

Experiment No. 2: Growth. The rate of growth was studied in the same groups of rats as reported in Experiment No. 1, but a further control group, consisting of intact animals, which received injections of solvent only, was added to provide data about the normal rate of growth. There were thus two control groups: adrenalectomized controls and intact controls. The rats were weighed every day throughout the experiment and the results were computed as the mean daily increment of weight.

Daily administration of Cl–F, 10 and 100 μg, or F–F, 100 μg, permitted a rate of growth analogous to that of untreated intact rats. Smaller doses of any of these compounds produced a growth rate which was less than

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Table I
Doses and mean response data (as percentage of control) of bio-assay on Na^+/K^+

Steroid (regression line)	Dose μg	Total N*	k**	Mean response	S.E.***
	0·01	11	2	82·39	12·48
	0·02	12	2	61·78	7·61
	0·05	20	3	56·34	6·32
Aldosterone	0·10	13	2	42·01	5·59
(y = 11·51–32·95 x)	0·25	6	1	28·29	6·88
	0·50	6	1	25·21	7·70
	0·068	—	—	*50*	—
	2	4	1	74·88	29·44
	5	20	3	49·13	6·81
DCA	10	26	4	45·10	5·67
(y = 83·11–39·30 x)	20	12	2	33·61	6·33
	7·00	—	—	*50*	—
	0·05	14	2	99·86	21·42
	0·10	11	2	83·59	18·42
F–F	0·25	13	2	75·04	15·72
(y = 43·79–43·77 x)	0·50	12	2	63·49	11·36
	1·00	6	1	37·07	12·80
	0·72	—	—	*50*	—
	0·05	7	1	79·47	19·93
	0·10	7	1	68·26	9·71
Cl–F	0·25	6	1	37·89	6·39
(y = 16·38–48·59 x)	0·50	5	1	35·58	7·38
	0·20	—	—	*50*	—

* N = No. of rats in dose assayed group. An equal number of rats was used as control for each group.
** k = No. of bio-assays.
*** Calculated as shown in Table II (2).

that of normal rats. Rats treated with 0·75 μg daily per rat of *aldosterone*, on the other hand, did gain weight when compared with the adrenalectomized control group, but it was far below the average daily increment shown by the intact rats.

Experiment No. 3: Na^+/K^+ bio-assay. Young adrenalectomized male white rats of an average weight of 75 g were used for this bio-assay. The procedure has been described in an earlier paper[1]. To interpret correctly the results it has to be taken into account that the activity of the material under test is indicated by the fall in the ratio of Na^+ to K^+ in the samples of urine excreted by the rats after the injection. This ratio is expressed as a percentage of the mean figure obtained for a control group on the same day of bio-assay. Thus a more intense effect is represented by a greater reduction of this percentage. Usually each dose of the materials has been assayed more than once and the grand mean and standard error given in Table I have been obtained by pooling the data belonging to each experiment.

It was found that there was a linear relationship between the Na^+/K^+ ratio expressed as a percentage of the control group and the logarithm of the dose in the following ranges; aldosterone: 0·01–0·50 μg; F–F: 0·05–1 μg; Cl–F: 0·05–0·50 μg (Table I).

Since no paired doses of two or more steroids were used on the same day of experiment, it was not possible

Table II
Compared relative activity of steroids on Na^+/K^+ $[X_{y=50}/X'_{y=50}]$

Steroid	Aldosterone	DCA	F–F	Cl–F
Aldosterone . .	—	100	10	3
DCA	0·01	—	0·1	0·03
F–F	0·1	10	—	0·27
Cl–F	0·34	35	3·6	—

to obtain an appropriate estimate of the potency ratio. Instead, to obtain a comparison of the relative potency of the materials the method of DESAULLES *et al.*[1] was followed. This involves the calculation and subsequent comparison of the dose which produces a 50% effect. In Table I this dose of each steroid has been calculated from the regression lines and in Table II the relative activities of the steroids have been accordingly arranged for comparison. These figures show the great activity of the halogenated steroids in modifying electrolyte excretion.

Hitherto no extensive report has been published concerning the electrolyte-controlling effect of the halogenated hydrocortisone acetates. BORMAN *et al.*[2] have found that Cl–F is 10 times as potent as, and F–F slightly more effective than, DCA. AXELRAD *et al.*[3] have reported that Cl–F shows 5·3 times the activity of DCA but this assessment is based only in the assay of two doses. The results of the present work (Table II) suggest that the halogenated compounds are much more potent. In this experiment aldosterone has proved to be 100 times as potent as DCA. This greater activity of aldosterone as compared with DCA is in agreement with previous data reported by several workers[4].

I wish to thank Prof. M. F. A. WOODRUFF for criticism of the manuscript; Prof. T. REICHSTEIN of Basle for a generous gift of aldosterone; and Dr. A. F. LANGLYKKE, Director of Research of The Squibb Institute for Medical Research, New Brunswick, N. J., for kind supply of halogenated steroids.

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Department of Surgery, Otago Medical School, Dunedin, New Zealand, April 18, 1955.

Zusammenfassung

Die Wirkungen von Aldosteron und 9 α-Halogenhydrocortison-azetaten auf das Überleben und Wachstum adrenalektomierter Ratten wurden studiert. Im Na/K-Ausscheidungstest zeigten sich 9 α-Chlor-hydrocortison-azetat bzw. 9 α-Fluor-hydrocortison-azetat 35- bzw. 10mal wirksamer als DCA und wiesen ⅓ bzw. $^1/_{10}$ der Wirksamkeit von Aldosteron auf.

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