At autopsy there were numerous proliferative areas on the gums, lips, and hard palate. The abomasum had numerous superficial ulcers 10–20 mm in diameter in the pyloric region. The pancreas was swollen, hard, and firm. The liver edges were rounded and slightly thickened. The distended gall bladder was filled with a dark, sticky, viscid bile, and the walls were thickened. Numerous mucous cysts were observed in the large bile ducts. The kidneys were enlarged, and numerous small subcapsular, clear cystlike structures were seen in the cortical portion.

Microscopically the cellular changes observed are similar to those seen in field cases and those reported by other workers. Marked keratinization of the hair follicles, with an excessive accumulation of keratinized material, along with a prolongation of the papillae of the skin, was noted. Central lobular degeneration of the liver cells, with bile duct proliferations, was evident. Dilation of the glands in the wall of the gall bladder was pronounced. In the kidneys, cystic dilation of the collecting tubules of the cortex, with a moderate degree of fibrosis, occurred. In the pancreas numerous areas of degenerating cells in the acini were also noted. A more complete and detailed report concerning these and other pathological changes is to be published later.

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Effect of Renin on Diuresis in Rats

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Croxatto *et al.* (*1*), using renin obtained from rat kidney, confirmed in this species the diuretic effect of renin that had been observed in rabbits by Pickering and co-workers (*2*) and under other conditions by Brandt *et al.* (*3*), Hughes-Jones *et al.* (*4*), Addis *et al.* (*5*), Mason *et al.* (*6*), Sellers *et al.* (*7*), and Barnafi (*8*). This study was undertaken to investigate the conditions that affect the influence of renin in the rat—e.g., the route of administration, the ingestion of NaCl, and adrenalectomy. Some of the experiments were carried out in normally hydrated animals with free access to water. In others the animals were hyperhydrated by forced administration of water, as described by Burn (*9*).

The normally hydrated animals (a total of 160), distributed in groups of 3 or 4 animals, were placed in metabolism cages, and the fluid consumption and the volume of urine excreted were measured every 12 hr. The results were recorded for periods of 10–40 days, depending on the experiment. The drinking fluid was either water or a 1% solution of NaCl. Renin and the other solutions administered were injected once a day intraperitoneally or subcutaneously, as indicated in Table 1.

The hyperhydration of rats was carried out under the conditions required for the Burn test (*9*). One group of rats was fasted with free access to water and then hyperhydrated by administering water through a gastric tube; the volume administered was 5% of body weight. The urine volume was recorded every 15 min. The injection of renin or other solutions was simultaneous with the hydration or preceded it by 1, 2, 3, 4, or 5 hr (Table 2).

Renin was extracted from pig, rat, or human kidney according to the techniques of Dexter (*10*) or Braun-Menéndez (*11*); 12.5–25 u of renin contained in 0.5–1 ml of solution was injected/100 g body weight. An inactive preparation (Ser. B) from pig kidney, as shown by its lack of pressor effect and its inability to produce hypertensin when incubated with hypertensinogen, was also tested. In some experiments (Ser. D, Table 1) hypertensin, at a dose of 1.5–9 u/rat, was injected in place of renin. Animals submitted to this treatment for more than 30 days were killed, and the hypertensinogen content of their blood was determined.

In normally hydrated rats drinking tap water (Ser. A, Table 1) the intraperitoneal administration of renin considerably increased urinary excretion, whereas the same dose of renin given subcutaneously modified it slightly or not at all (Fig. 1).

The stimulating effect on diuresis is observed after each injection of renin, but it decreases with repeated injections. This behavior could be explained by the formation of antirenin on prolonged administration of renin. In agreement with this assumption, we found that the serum of these rats does not produce hypertensin when incubated with renin, but produces pepsitensin when incubated with pepsin. This indicates that there is no lack of substrate (hypertensinogen), but that an inhibitor of renin is present.

Inactive kidney extracts never stimulated diuresis, no matter what the dose or the route of administration (Ser. B, Table 1).

The diuretic action of renin is considerably increased in animals drinking 1% NaCl (Fig. 1). Pronounced effects are observed even when renin is administered subcutaneously (Ser. C, Table 1).

Hypertensin does not show a diuretic effect even at a dose of 9 u; only weak and irregular effects were obtained with larger doses given subcutaneously (Ser. D, Table 1).

Adrenalectomy in rats drinking 1% NaCl considerably decreased or suppressed the stimulating effect on diuresis produced by daily intraperitoneal injections of renin. A dual effect of renin was observed in the polyuria of normal hyperhydrated animals

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TABLE 1

EXPERIMENTS ON NORMALLY HYDRATED RATS

Series	Group*	Drinking fluid	Injected with	Route of injection	Intensity of diuretic effect§
A—normals	a	Water	Renin†	Intraperitoneal	++
	b	"	"	Subcutaneous	0
	c	"	0.9% NaCl	Intraperitoneal	0
B—normals	a	Water	Renin	Intraperitoneal	++
	b	"	Inactive renin	"	0
C—normals	a	1% NaCl	Renin	Intraperitoneal	+++
	b	"	"	Subcutaneous	+
	c	"	0.9% NaCl	"	0
D—normals	a	Water	Hypertensin‡	Intraperitoneal	0
	b	"	"	Subcutaneous	0 + (?)
E—adrenalectomized	a	1% NaCl	Renin	Intraperitoneal	0
	b	"	0.9% NaCl	"	0

* Each group composed of 3 rats.
† 12.5–25 u of renin injected/100 g body weight.
‡ 4.5 u of hypertensin injected/100 g body weight.
§ 0 indicates no effect in the 12 hr following injection ; + indicates the intensity of the diuretic effect by comparison with the periods in which the animals were not injected with renin.

(Ser. F, Table 2). Water excretion is slowed down when the overloading with water is produced immediately after or within 1 hr following the intraperitoneal injection of renin. This antidiuretic effect decreases rapidly, and in rats hyperhydrated 2, 3, and 5 hr after the injection the effect is reversed, resulting in a considerable and progressive increase in water excretion (Fig. 2).

In adrenalectomized rats (Ser. G and H, Table 2) hyperhydrated 2 and 5 hr after intraperitoneal injection of renin, a marked delay in water excretion is observed as compared to normal animals under the same conditions or to adrenalectomized rats injected with 0.9% NaCl instead of renin (Fig. 3). Some of the adrenalectomized animals, drinking 1% NaCl for a prolonged period, show a more active diuresis when hyperhydrated 2 hr after renin injection, but never reach the high level of excretion of normal animals.

Renin was injected into rats (adrenalectomized 20–30 days before, Ser. H. Table 2) 2–4 days after the drinking of 1% NaCl solution was discontinued. The

TABLE 2

EXPERIMENTS ON HYPERHYDRATED RATS

Series	Group*	Drinking fluid	Intensity of diuretic effect†
F—normals	0	Water	—
	1	"	—
	2	"	(±) +
	3	"	+++
	4	"	++
G—adrenalectomized (5 days)	0	NaCl	—
	1	"	—
	2	"	— + (?)
	3	"	—
	4	"	—
H‡—adrenalectomized (30 days)		NaCl and water	
	0		—
	1	" " "	—
	2	" " "	—
	3	" " "	—

* Each group composed of 3 rats. The number of the group indicates the time (in hr) after intraperitoneal injection of renin (12.5 u/100 g body weight) at which the animals were hydrated. The amount of water given by stomach tube was 5% of body weight. The controls injected with 0.9% NaCl and "inactive" renin are not shown in the table.
† 0, +, and – indicate whether the volume of urine excreted was the same or greater or lower than in the animals injected with 0.9% NaCl.
‡ Composed of rats adrenalectomized 30 days before the experiment and drinking 1% NaCl solution for 27 days, followed by tap water.

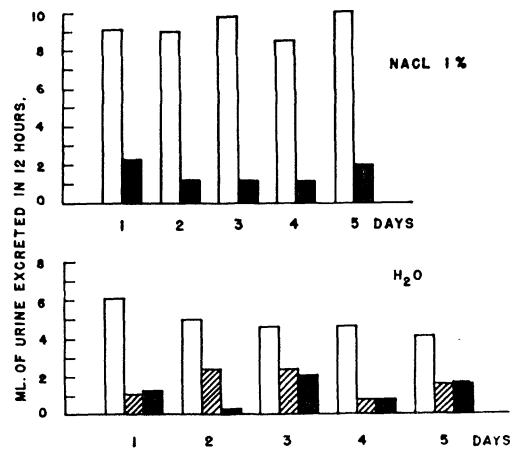

FIG. 1. Urine volume (ml) per 100 g body weight and 12 hr. in the first 5 days of the experiment. Each bar represents the average for 6 rats. Upper part of graph refers to animals drinking 1% NaCl. White bars refer to rats injected intraperitoneally daily with 0.5 ml pig renin/100 g body weight, and the black bars to those injected with 0.9% NaCl. Lower portion of graph refers to diuresis of rats drinking tap water. White bars refer to rats injected intraperitoneally with 0.4 ml renin/100 g body weight, hatched bars to those that received the same dose subcutaneously, and black bars to those injected intraperitoneally with 0.9% NaCl.

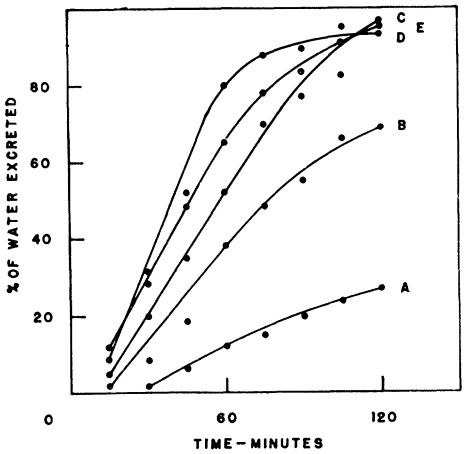

FIG. 2. Percentage of urine volume excreted by 4 normal rats as related to the amount of water administered by stomach tube (5% of body weight, Burn test [9]). All animals were injected intraperitoneally with 0.5 ml renin/100 g of body weight. *A* corresponds to the group of rats simultaneously injected and hydrated; *B*, to the group hydrated 1 hr after renin injection; *C*, 2-hr interval; *D*, 3-hr interval; *E*, 4-hr interval.

injection was given 2 hr before water overloading and was followed by a marked antidiuretic effect. This effect was greater than that observed in normal rats injected with the same dose of renin simultaneously with water overloading.

The results obtained with normally hydrated animals (Ser. A, B, and C, Table 1) demonstrate the effects of renin on water metabolism and urinary excretion, which depend on the route of administration and the amount of NaCl in the drinking water. These effects appear to be related to the ability of renin to react with hypertensinogen, but hypertensin

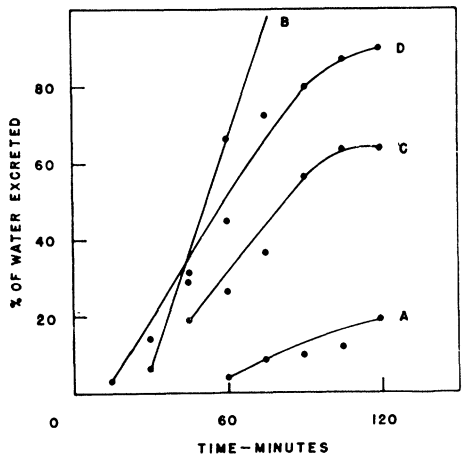

FIG. 3. Percentage of urine volume excreted by normal and adrenalectomized rats, as related to the amount of water administered by stomach tube (5% of body weight, Burn test [9]). *A* corresponds to 4 adrenalectomized rats, injected with 0.5 ml renin/100 g body weight; *B*, normal rats injected with same dose of renin; *C*, 4 adrenalectomized rats injected with 0.9% NaCl; *D*, normal rats injected with 0.9% NaCl. All animals injected intraperitoneally with renin or NaCl 2 hr prior to water administration by stomach tube. Normal and adrenalectomized rats drank 1% NaCl for 4 days before injection.

does not seem to be the responsible factor (Ser. D, Table 1).

The greater diuretic effect of renin on the rats drinking NaCl solution is a confirmation of the probable relation of this effect with sodium excretion (*2, 7*).

The absence or slight stimulation of diuresis by renin on adrenalectomized rats with free access to water (Ser. E, Table 1) is a demonstration of the importance of the integrity of the adrenal function for this effect. The results obtained in hyperhydrated rats is a confirmation of the same findings (Ser. G, Table 2).

The analysis of the results of the studies on water excretion in normal rats hyperhydrated at different intervals after renin injection indicates that the effects on diuresis are complex. Two phases may be considered. The immediate effect, lasting ½–1 hr, is an inhibition of diuresis, followed by a more prolonged phase from the second to the fifth hour after intraperitoneal injection of renin, characterized by an acceleration of water excretion.

In adrenalectomized hyperhydrated rats, particularly those deprived of NaCl, renin shows only the antidiuretic effect lasting several hours, indicating that intact adrenal function or a normal NaCl balance is required for the diuretic action to appear.

The increase in diuresis produced by renin is independent of the changes in blood pressure. The intense effect of renin injection on diuresis supports the views of Fasciolo (*12*) and Brandt *et al.* (*3*) in attributing a more important role to this kidney enzyme in water metabolism. On the other hand, more recent studies seem to indicate that a closer relationship exists between the hormonal hypertensive mechanism and water and NaCl balance. Renin might be a common factor for both processes.

We cannot explain the difference in action of renin when given intraperitoneally or subcutaneously; but it is possible that, in addition to a difference in the rate of absorption or destruction of renin, some intrahepatic mechanism may be at work when renin is given intraperitoneally. It is difficult to decide at present whether the antidiuretic and diuretic effects of renin are due to the enzyme itself or to impurities, or to some other specific substance produced by the enzyme, as is the case for the antidiuretic factor obtained in the reaction of hypertensinogen with pepsin (*13*). In any case, hypertensin is not responsible for the polyuria produced by renin when given intraperitoneally to the rat. The possible influence of renin on the secretory mechanisms of the hypophysis and the adrenal are under study.

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Mapping Functions in Tetrad and Recombinant Analysis

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In the ascomycetes and basidiomycetes, all four products of a single meiosis can be isolated and characterized. Data so obtained possess obvious advantages in precision and statistical efficiency over those provided by the usual genetic material, which involves random sampling of single strands. In particular, the analysis of tetrads offers a sensitive method for examining the details of the meiotic process.

Various methods have been proposed for employing tetrad data in the mapping of loci. Whitehouse (*1*) and Mather and Beale (*2*) have provided a careful and exhaustive analysis of the information available in those cases (e.g., *Neurospora crassa, Bombardia lunata*) in which the linear order of the segregants is known.

The problem of mapping in the more general and widespread situation of unordered spore arrays has been considered by Lindegren (*3*), who developed a graphical method.

A less cumbersome and inherently more accurate approach was made possible by an analytic solution to the problem. To aid in the subsequent discussion we shall adopt the following symbolism: P_{AO}, P_{BO}, etc., will denote the frequencies of M II segregations of the loci, *A, B,* etc. which result from crossovers occurring between the locus concerned and its centromere; P_{AB} will signify the frequency of M II segregations of A and B to yield tetratype asci; p_{AB} will denote the recombinant frequency between A and B.

Perkins (*4*) and Whitehouse (*5*) noted that in the cross $AB \times ab$, the frequency of tetratype asci (i.e., AB, Ab, aB, ab) is given by

$$P_{AB} = P_{AO} + P_{BO} - \frac{3}{2} P_{AO} P_{BO}, \qquad (1)$$

if A and B are independent. Perkins (*4*) pointed out that with the aid of another locus, C, not linked to either A or B, equations analogous to (1) can be written for P_{AC} and P_{AB}. The resulting set of three simultaneous equations may then be solved for P_{AO}, P_{BO}, and P_{CO}, as was done by Whitehouse (*6*). The three genes can thus be localized with reference to their respective centromeres in terms of their M II segregation frequencies.

It is perhaps worth noting that this procedure cannot be applied if two of the three loci are linked and in the same arm. Thus, if A and B are so linked, the three probabilities P_{AB}, P_{AC}, and P_{BC} are no longer independent and one obtains instead of (1)

$$P_{AB} = \frac{P_{BO} - P_{AO}}{1 - \frac{3}{2} P_{AO}}, \qquad (2)$$

which does not form a solvable set of simultaneous equations with the other two relations that can be written for P_{AC} and P_{BC}.

Data obtained by the proper application of these or analogous methods can provide consistent information in terms of distances from the centromere. Difficulty arises, however, when it becomes necessary to compare such map distances with those obtained by the conventional method, which depends on the frequency of recombinant strands. It has been assumed by the authors mentioned above, as well as by others, that

$$\mathfrak{p}_{AB} = \frac{1}{2} P_{AB}, \qquad (3)$$

where, as above, $\mathfrak{p}_{AB}$ refers to recombinant frequency between A and B and P_{AB} the corresponding M II frequency.

The reasoning often offered to justify this conversion is that only one half of all crossovers that occur are observed in ordinary recombinant analysis, since only one chromatid out of any given tetrad is recovered. Although they employ this conversion factor, both Rizet and Engelmann (*7*) and Papazian (*8*) have pointed out that it at best represents an approximation which can be valid only over short map distances. That this contention is correct is evident from the fact that the limit approached by $\mathfrak{p}_{AB}$ as the number of chiasmata between A and B increases is ½, whereas the limit of P_{AB} is $\frac{2}{3}$. Thus for long map distances relation (3) would yield a value of 0.33 for p_{AB} instead of the 0.5 to be expected.

The accurate conversion of M II frequencies into recombinant map units requires the derivation of the explicit relation between P_{AB} and $\mathfrak{p}_{AB}$. A relation of this kind can be obtained from the corresponding mapping functions. In the absence of chiasmata interference, these functions can be simply deduced, since for any fixed average the number of chiasmata will be distributed according to the terms of the Poisson series. Haldane (*9*) has shown that under these conditions the frequency of recombinant strands is given by

$$p_{AB} = \frac{1}{2} (1 - e^{-2m'}) \qquad (4)$$

where m' is the *average* number of chiasmata per *two strands* occurring between the relevant loci. Mather (*10*) has demonstrated that the proportion of a set of tetrads which will exhibit M II segregation if each tetrad has experienced *precisely* r chiasmata between A and B is given by