ported.[3] ‡ For the immunized animals the ratio between HD50 and LD50 is in the order of 1:1, all doses smaller than one LD50 failing to produce hemorrhage. It is evident that immunization with the endotoxin preparation used protects against both tumor-hemorrhage induction and primary lethality; also that the relative effectiveness of immunization is very much greater in the case of hemorrhage induction than in the case of primary lethality.

After immunization the HD50 of the antigen is of about the same magnitude as the LD50, yet for the non-immunized tumor-bearing animals the ratio between HD50 and LD50 is 1:200. Hence it would seem that the neutralizing action of the antibody must be prompt and complete, since but a very small quantity of uncombined antigen would be sufficient to induce tumor hemorrhage. In this respect the protection provided by immunization appears to differ from that afforded by sulfanilamide in that immunization protects against two hundred HD50, whereas sulfanilamide protects against only sixteen HD50.[6] Evidently, and as one would suppose, the mode of antitoxic action of sulfanilamide is of a different nature from the immunological mode of protection.

The preceding papers of this series suggest a uniformity in constitution and in toxic action of the complex O antigens of nearly all gram negative bacteria. The widespread occurrence among gram negative bacteria of the tumor-hemorrhage incitant and its correlation with the occurrence of O antigens was adduced as evidence for the existence of a single uniform toxin in the O antigens of gram negative bacteria.

The present paper describes the type of antitoxic immunity obtained when the same organism is used for both immunization and hemorrhage production. Cross-immunization experiments, using gram negative organisms unrelated except for the exhibition of common tumor hemorrhage production—a highly sensitive method of detecting these endotoxins—is expected to furnish critical evidence as to the unitary character of these toxins. Such experiments are now in progress.

Conclusion. Tumors implanted in mice previously immunized with *Shigella paradysenteriae* Flexner were found, in contrast to those implanted in non-immunized control mice, to be highly resistant to the tumor-hemorrhage factor of the Flexner organism.

‡ It is to be noted that in the case of highly purified or possibly dissociated antigen the HD50/LD50 dosage ratio may be as great as 1:5000.[3,8]

[8] Shear, M. J., *Cancer Research*, 1941, **1**, 731.

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Activity of Parathyroid Hormone in the Nephrectomized Rat.

HERBERT C. STOERK. (Introduced by A. M. Pappenheimer.)

From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City.

The question of whether some of the effects of parathyroid extract injections are producible in the absence of the kidneys has been studied by several investigators, in the effort to decide if parathormone acts primarily upon the kidneys.

Collip and others[1] found that the injection of 80 units of PTH into bilaterally nephrectomized rats produced the customary changes in the bones.

This has been confirmed and the observation recently extended by Selye[2] who showed that nephrectomy alone produces a mild degree of "osteitis fibrosa" which was prevented by parathyroidectomy.

[1] Collip, J. B., Pugsley, L. I., Selye, H., and Thomson, D. L., *Brit. J. Exp. Path.*, 1934, **15**, 335.

[2] Selye, H., *Arch. Path.*, 1942, **34**, 625.

Ellsworth[3] observed an elevation of the serum Ca after injection of 700 units of PTH into nephrectomized dogs.

Tweedy and his coworkers[4,5] also working on nephrectomized dogs, could not detect a significant rise in serum Ca after injection of 100-250 units of PTH.

Recently Neufeld and Collip[6] reported that they were unable to obtain an elevation of the serum Ca after PTH injections into nephrectomized and ureter ligated rats, cats and dogs. There was, however, no comparison made with nephrectomized uninjected animals.

The difficulty in raising the serum Ca in animals deprived of their kidneys, is obvious. Considering the well known Ca-P antagonism and the fact that the serum phosphate in these animals is found to be several times higher than normal, it cannot be expected that animals without kidneys would respond like normal animals to PTH injections. Even in comparing injected and uninjected nephrectomized animals, there is still a possibility of misinterpretation. The fact that renal insufficiency leads to parathyroid hyperfunction is well known[7] and one may expect that in the uninjected nephrectomized animals used for comparison the parathyroids are hyperactive. Since the effect of PTH injections on the activity of the parathyroids is unknown, a second variable may be introduced when the experimental findings in the nephrectomized, PTH injected animals are compared with those in the uninjected nephrectomized controls. On reinvestigating the problem we wished to avoid this possibly improper comparison by removing both kidneys and parathyroids in the PTH injected and uninjected group. We also attempted to detect the extent of a possible parathyroid hyperactivity in the nephrectomized rat by its action on the serum Ca concentration.

Experiments. For our experimental series, 47 male albino rats were selected from our stock colony and divided into 9 groups of 5 to 6, using litter mates when possible. They ranged in age from 15-17 weeks and in body weight from 280-350 g. The animals were kept on our stock diet (Rockland pellets). Ether anesthesia was used for all operations and for killing the animals at the end of the experiments. One group of 6 animals served as normal controls, 6 were parathyroidectomized, 5 normal unoperated rats were injected with 5×20 units of PTH (Lilly extract) subcutaneously.* Two groups of 5 animals were nephrectomized; one group was continued on the stock diet, in the other food was withdrawn after the operation. Two groups of 5 animals were nephrectomized and immediately afterwards, parathyroidectomized, one group fasted after the operation. Two further groups of 5 parathyroidectomized-nephrectomized animals under the same conditions as the previous groups were injected subcutaneously with 5×20 units of PTH. All animals were killed 48 hours after the operations and first injections. The last injection was given 4-6 hours before the animals were killed. Blood was then taken from the right auricle and the serum analyzed in individual samples for Ca (Clark-Collip) and phosphates (Kuttner-Cohen). The total serum protein was determined in the nephrectomized groups with the refractometer. Bones were examined histologically.

Findings. The results are given in the table. The nephrectomized fasted and non-fasted groups were combined since no significant differences in the serum levels were found. The individual serum Ca values in the 3 nephrectomized groups are seen in the scatter chart. The experimental findings in the table have been arranged in 2 sections. In the first section is shown the effect of parathyroidectomy after 48 hours upon the serum Ca and phosphate in non-nephrectomized and nephrectomized animals. The second

[3] Ellsworth, R., and Futcher, P. H., *Bull. Johns Hopkins Hosp.*, 1935, **57**, 91.

[4] Tweedy, W. R., Templeton, R. D., and McJunkin, F. A., *Am. J. Physiol.*, 1936, **115**, 514.

[5] Tweedy, W. R., *Endocr.*, 1937, **21**, 55.

[6] Neufeld, A. H., and Collip, J. B., *Endocr.*, 1942, **30**, 135.

[7] Pappenheimer, A. M., *J. Exp. Med.*, 1936, **64**, 965.

* We are greatly indebted to the Ely Lilly Company for the generous supply of parathyroid extract.

TABLE I.

		No. of animals	Serum Ca Avg	Serum Ca Range	Inorganic serum PO_4 Avg	Inorganic serum PO_4 Range
I	*Non-nephrectomized*					
	Normal control	6	10.7	10.3-11.0	7.7	7.3-8.2
	Parathyroidectomized	6	8.3	6.6-9.7	9.0	8.0-9.5
	Nephrectomized					
	Non-parathyroidectomized	10	9.6	8.6-11.0	14.6	10.1-17.1
	Parathyroidectomized	10	6.5	5.3-7.1	14.5	12.1-18.1
II	*Non-nephrectomized*					
	Normal control	6*	10.7	10.3-11.0	7.7	7.3-8.2
	Injected	5	12.3	11.9-12.5	8.3	7.9-8.7
	Nephrectomized					
	Parathyroidectomized control	10*	6.5	5.3-7.1	14.5	12.1-18.1
	Parathyroidectomized injected	10	10.5	8.8-11.9	15.3	14.1-16.9

* Same animals as in first section.

section serves the purpose of comparing the effect of PTH injections in the normal and double operated rats. It is seen from the data that after 48 hours parathyroidectomy alone lowers the serum Ca from

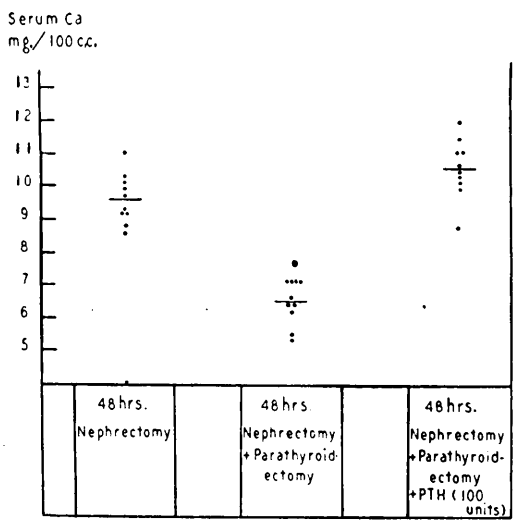

FIG. 1.

10.7 to 8.3 mg %. It increases the serum phosphate from 7.7 to 9.0 mg %. In the nephrectomized groups the serum Ca is 3.1 mg % lower after the combined operation than in those in which only the kidneys were removed. The serum phosphate values show no differences between the two groups. This clearly indicates that the parathyroids are able to maintain a relatively high blood Ca level in absence of the kidneys. The first 2 groups in the second section of the table demonstrate the well known serum Ca elevating effect of PTH injections in non-nephrectomized animals. The injection of 5×20 units of PTH produces after 48 hours an average rise in the serum Ca of 1.5 mg % without affecting the serum phosphate. The latter finding is in accordance with Collip's data. In the absence of the kidneys and parathyroids the same dosage produces an elevation of the serum Ca to 10.5 mg % compared with 6.5 mg % in the uninjected controls. The serum phosphate again is unchanged.

Thus in the absence of the kidneys, the blood Ca is maintained at a normal level because of the increased activity of the intact parathyroids; when these are removed, the blood Ca can be elevated by the injection of PTH.

The average total serum protein did not significantly differ within the 3 nephrectomized groups; it averaged 6.61 mg % in the nephrectomized animals, 5.91 mg % in the double operated and 6.22 mg % in the double operated injected group. Thus hemo-dilution or hemoconcentration is not likely to account for the differences in the blood Ca level. The histology of the bone, comparing the distal epiphysis of the right femur, was in accordance with the findings of Selye. "Osteitis fibrosa" was absent in the double operated uninjected group, mild in the nephrectomized

and pronounced in the double operated injected animals.

Summary. Within 48 hours, parathyroidectomy lowers the serum Ca in non-nephrectomized mature rats from 10.7 to 8.3 mg %. In the same period of time the serum Ca in nephrectomized-parathyroidectomized rats decreases from 9.6 to 6.5 mg %.

The injection of 5×20 units of PTH into normal rats, after 48 hours produces an average elevation of the serum Ca of 1.5 mg %. The injection of the same quantity of PTH into parathyroidectomized-nephrectomized rats, after the same time produces an average elevation of the serum Ca of 4 mg % (from 6.5-10.5 mg %). Although these experiments prove that PTH raises the blood Ca without the intermediation of the kidneys, they do not exclude the possibility that the hormone may under normal conditions also affect renal function.

Conclusion. (1) The serum Ca elevating activity of the parathyroid hormone is demonstrable in bilaterally nephrectomized rats. (2) Confirming previous observations, it has been found that "osteitis fibrosa" is produced by PTH injection in the absence of the kidneys.

The author wishes to express his thanks to Mrs. Claudia Schogoleff for her technical assistance.

Since this paper was submitted, there has appeared a paper by Ingalls, Donaldson and Albright (*J. Clin. Invest.*, 1943, **22**, 4) showing that injections of PTH in nephrectomized rats produces more severe skeletal lesions than those which result from nephrectomy alone.

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B Vitamins in Germinating Seeds.*

VERNON H. CHELDELIN† AND ROBERT L. LANE. (Introduced by R. Wulzen.)
From the Department of Chemistry of the University of Texas, Austin, Texas.

In the course of studies on the B vitamin contents of foods, it was noticed that samples of fresh corn, peas and beans contained greater amounts of certain B vitamins than did the dried seeds. This suggested that the B vitamin levels in these seeds might be related to their metabolic activity.

Germination of various seeds is known to be accompanied by increases in ascorbic acid and thiamin content.[1-4] Experiments were therefore begun to determine whether this was also true for the other B vitamins.

Samples of blackeyed peas, lima beans and cotton seeds were allowed to germinate in covered Petri dishes between layers of moist filter paper. The temperature was maintained at approximately 30° for periods of 36 and 48 hours. At the end of the germination period the seeds were ground, digested with takadiastase and papain according to the general enzyme procedure of Cheldelin, Eppright, Snell and Guirard,[5] and assayed for 8 B vitamins. Microbiological methods of assay were employed throughout.[6] The results are shown in Table I.

Comparisons are made on the dry weight basis, in order to compensate for differences in moisture content. Although many samples which absorb large amounts of water during germination do not appear to gain in

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† Present address, Department of Chemistry, Oregon State College, Corvallis, Oregon.

1 Biswas, H. G., and Das, K. L., *Science and Culture*, 1938, **4**, 360.

2 Muthanna, M. C., and Ahmad, B., *Current Sci.*, 1940, **9**, 320.

3 Mentzer, C., *Bull. soc. chim. biol.*, 1940, **22**, 444.

4 Rytz, W., Jr., *C. R. Soc. Biol.*, 1938, **129**, 814.

5 Cheldelin, V. H., Eppright, M. A., Snell, E. E., and Guirard, B. M., *The University of Texas Publication*, 1942, No. 4237, 15.

6 Williams, R. J., *The University of Texas Publication*, 1941, No. 4137, 7.