tumor strain 38, used in previous co-carcinogenesis studies with Bittner's virus (MTV), now appears to carry this virus and causes mammary tumors in adult mice of a strain (LAF_1) in which spontaneous mammary tumors are very rare. This strain has marked mammary gland stimulating, marked adrenotropic and moderate growth-promoting activities. 2. Numerous similar MtT strains studied earlier failed to induce mammary tumors. 3. Electronmicrographs showed MTV-like particles in both mammotropic tumor and mammary gland. 4. In a small experiment no mammary tumors developed in bilaterally adrenalectomized mice bearing this mammotropic tumor, even though adrenalectomy enhanced the growth of the mammotropic tumor. Prevention of induction of mammary tumor by adrenalectomy might be related to inhibition of milk secretion and reduction of viral concentration. 5. Cancerization by this mammotropic tumor is extraordinary so that almost every mammary pad examined had microscopic mammary carcinomas (both glandular and squamous) and anaplastic (presumably precancerous) changes.

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Enhancement of Metastatic Calcification Produced with Parathyroid Extract by Parathyroidectomy and Adrenalectomy. (28778)

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The present experiments were prompted by the following observations: During attempts to maintain parathyroidectomized (PTX) rats on calcium lactate and daily injections of 50 units of bovine parathyroid extract (PTE), it was noted that this treatment caused death associated with widespread calcific changes within several days. Similarly treated intact rats survived for many weeks and exhibited only mild changes. Adrenalectomized (ADX) rats were also seen to tolerate less PTE than their intact controls. Attempts were made to quantitate the calcific changes with the aid of radiocalcium.

Methods. Male Holtzman rats fed Purina Laboratory Chow and weighing from 125 to 150 g were used in all experiments. Parathyroidectomies, by electrocautery, and adrenalectomies were performed under ether anesthesia. All operated rats, as well as their controls, were placed on a 2% calcium lactate

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TABLE I. Effect of Parathyroidectomy and Adrenalectomy upon "Nephrocalcinosis" Produced by Parathyroid Extract.

Group	No. of rats	PTX	ADX	DOCA (mg)	Compound F (mg)	Counts/min/mg (avg ± S.E.)
1	50	—	—	—	—	43 ± 8
2	50	+	—	—	—	343 ± 38
3	45	—	+	—	—	225 ± 14
4	31	—	+	1.0	—	137 ± 15
5	10	—	+	3.0	—	90 ± 13
6	10	—	+	9.0	—	157 ± 33
7	23	—	+	—	4.0	104 ± 21
8	37	—	+	1.0	4.0	38 ± 5
9	10*	—	—	—	—	17 ± 1

* Starved controls.

solution instead of drinking water and were injected subcutaneously 3 times with 50 units of PTE (Eli Lilly). The first injection was given at start of the experiment, the second 6 hours later, and the third and final injection 24 hours after the first. Hydrocortisone acetate† and desoxycorticosterone acetate (DOCA)‡ were given subcutaneously in 2 doses, one at the time of initial injection of PTE and the second with the final injection of PTE. The rats were killed 5 hours after the last injection of PTE. One hour before sacrifice all animals were injected intravenously with a standard dose of $Ca^{45}Cl_2$ (5 million counts/minute) in 0.5 to 1.0 ml saline.

Hearts and kidneys were removed and weighed, and the calcium extracted with 5 ml of 6 N HCl for 18 hours at 90°C. The acid extract was diluted to 25 ml with distilled water and filtered. A 0.2 ml aliquot was counted in a toluene-ethanol-phosphor mixture in a Tri-Carb Liquid Scintillation Counter.

In some of the experiments, before weighing and extracting, sections of heart and kidney were taken for histological examination after hematoxylin-eosin and von Kossa staining.

Results. The extent of calcific changes in heart and kidney were judged from von Kossa stained sections and from the concentration of Ca^{45}/mg of tissue. Since in the various experimental groups there was essentially no discrepancy between the intensity of the calcific changes in kidney and in heart, Ca^{45} content was determined in most cases from renal tissue alone.

Fig. 1 illustrates the correlation between isotopic measurements and extent of nephrocalcinosis in von Kossa stained sections of the same kidney. Normal rats injected with $Ca^{45}Cl_2$ alone retained 10.3 ± .15 counts/minute/mg kidney. In PTE-injected rats, from 10 to 50 cpm/mg of kidney were not associated with significant calcification detectable by the silver stain. Readily recognizable calcium deposits were evident in kidneys containing about 100 cpm/mg tissue. More pronounced calcific lesions were associated with higher Ca^{45} concentrations in the renal extracts. It appeared, therefore, that by means of introducing labeled calcium, the extent of calcific changes in tissues could be judged in a quantitative fashion without relying on grading of gross or microscopic observations.

Experiments in which widespread renal necrosis was produced with the aid of kanamycin showed that early tubular necrosis without calcification was not associated with increased accumulation of the isotope. Later, with the appearance of calcium deposits in the necrotic lesions, the Ca^{45} content of extracts of renal tissue increased as did the amount of discernible silver staining material in sections.

The findings are summarized in Table I. Comparison of the first 2 groups illustrates that functioning parathyroids afford striking protection from excess bovine PTE. Similarly, some activity of the adrenal glands is seen to prevent the calcific changes produced by PTE (Groups 1 and 3). Either

† Merck & Co., Inc., 'Hydrocortone.'

‡ Schering Corp., 'Cortate.'

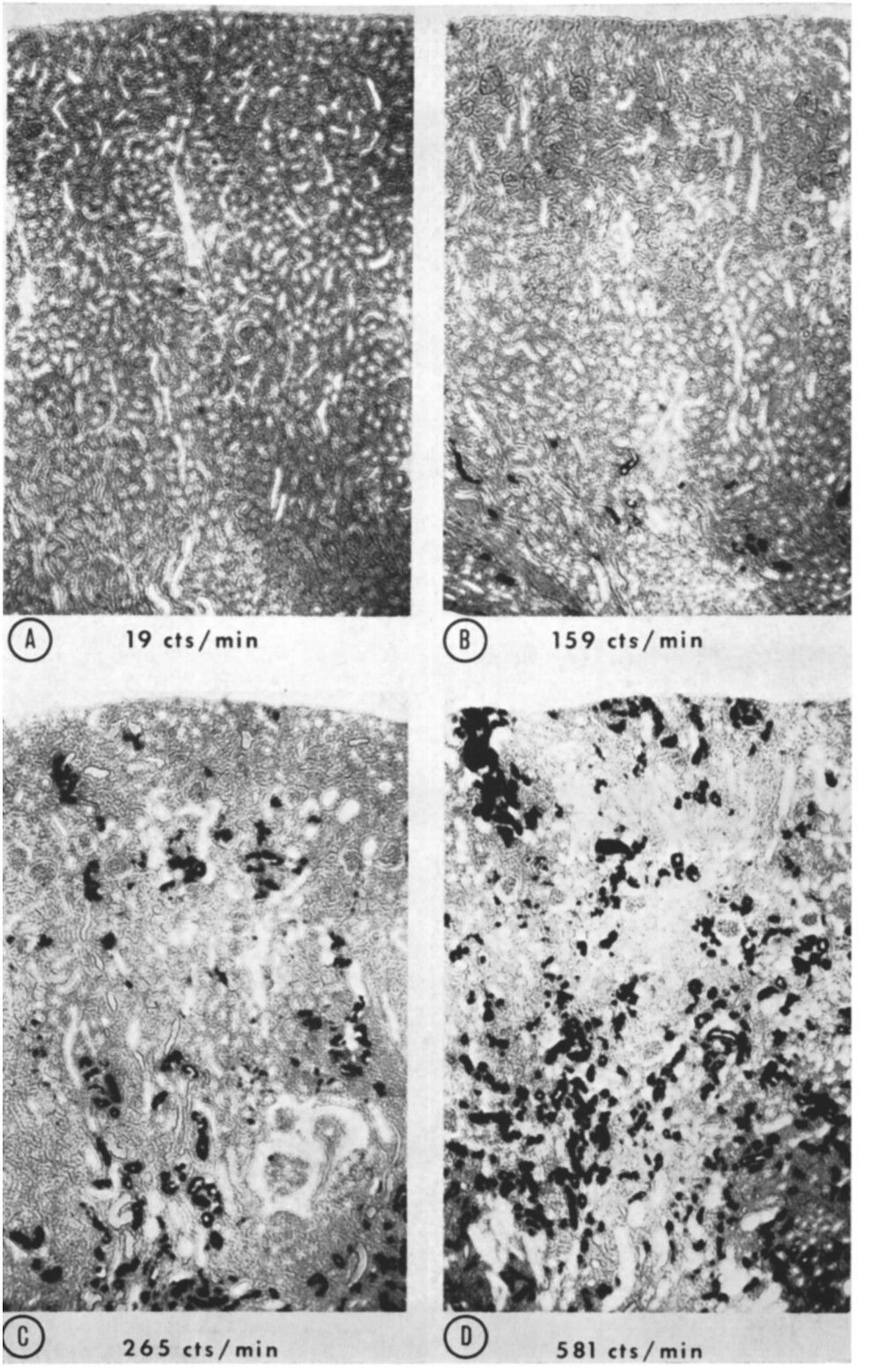
A
19 cts/min
B
159 cts/min
C
265 cts/min
D
581 cts/min

FIG. 1. Comparison of sections of kidney stained after von Kossa with Ca^{45} content (cpm/mg kidney) in the same kidneys. Microphotographs illustrate kidneys from rats of groups listed in Table I. (A—Group 9; B—Group 4; C—Group 3; D—Group 2.)

DOCA (Groups 4-6) or hydrocortisone (Group 7) appeared to diminish the calcific changes. However, treatment with both steroids (Group 8) was required for complete protection. In contrast to the reports of Lehr(1), no potentiation of PTE toxicity was observed even with very large doses of DOCA (Group 6). Loss of body weight as it occurred in Groups 2 and 3 was found not to be a contributing factor, since intact rats given no food but offered the 2% Ca-lactate solution during the experimental period, showed comparable weight losses but exhibited no detectable calcific changes (Group 9). It appeared, therefore, that protection from calcific changes depended on the integrity of parathyroids and adrenals alike.

It was also seen that Ca intake was not related to the development of the calcific lesions. PTE-injected PTX rats ate only half as much diet and drank 40% less of the Ca-lactate solution than PTE-injected intact controls. Thus, in spite of a greatly lowered dietary intake of Ca, the intensity of the calcific lesions was greatly enhanced. An explanation for this finding was sought and apparently found from a comparison of the serum Ca levels in these 2 groups. Serum Ca levels in 15 PTE-injected PTX rats at the end of the experiment were 15.6 $\pm$ 0.27 mg% (serum P: 9.0 $\pm$ 0.16 mg%), in 15 PTE-injected intact rats 13.3 $\pm$ 0.27 mg% (serum P: 8.4 $\pm$ 0.14 mg%). It thus appears that in the absence of functioning parathyroids the blood Ca-elevating activity of exogenous PTS is strikingly enhanced.

Discussion. From a body of circumstantial evidence, the existence of a blood Ca-lowering hormone in parathyroid has recently been suggested by Copp(2). While other explanations appear possible, the strikingly reduced resistance of PTX rats to bovine PTE may be due to a lack of Copp's "calcitonin."

Lehr(1) has studied the incidence of calcific cardiovascular lesions in rats with secondary hyperparathyroidism due to renal injury. As expected, he observed that parathyroidectomy prevented the appearance of the lesions. He also concluded that following adrenalectomy the calcific changes failed to develop. However, since in these experiments ADX rats succumbed earlier than their controls, it appears equally possible that adrenal insufficiency contributed merely by reducing the time of exposure to excess PTH. Conversely, in experiments with adrenalectomized, nephrectomized, parathyroidectomized rats, DOCA was interpreted to enhance the calcific changes caused by PTE. However, DOCA-treated rats lived longer and were more frequently injected with PTE than their controls.

A number of recent observations suggest an antagonism of adrenal cortical activity against PTH. While in the presence of functioning parathyroids, adrenal insufficiency is not associated with any change in blood Ca, parathyroidectomized rats respond to adrenalectomy with a rise in blood Ca(3). Conversely, it was shown that during parathyroid insufficiency, hydrocortisone lowers the blood Ca of man(4), dog and cat(5) and rats(3). Furthermore, the existence of an antagonistic action of hydrocortisone has been established with regard to the effects of PTA upon serum Ca(6) and the morphology of bone(7). The enhancement of PTE toxicity during adrenal insufficiency and its reversal by DOCA and hydrocortisone appear in keeping with these observations.

Summary. Groups of rats were injected with 150 units of bovine PTE over 28 hours. At the end of experiment they were injected intravenously with a standard dose of $Ca^{45}Cl_2$. The extent of the calcific changes was judged from von Kossa stained sections and from concentrations of Ca^{45}/mg tissue. Striking enhancement of metastatic calcifications was observed in parathyroidectomized and in adrenalectomized rats. DOCA and hydrocortisone prevented the appearance of the calcific changes in adrenalectomized rats.

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Effect of Dietary Phenylalanine and Tryptophan on Pineal and Hypothalamic Serotonin Levels.* (28779)

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Serotonin (5-hydroxytryptamine) appears to be significant in normal brain function since its formation, levels and metabolism within the brain have been experimentally related to certain brain activities or their behavioral manifestations. Reduction in brain serotonin in rats occurs with a tryptophan-deficient diet(1) or increased dietary L-phenylalanine(2,3,4,5), and increase occurs with an increased intake of L-tryptophan(2). It is important, however, to examine these changes in anatomically and functionally discrete areas of the central nervous system, since their occurrence may not be general and their further physiologic and behavioral effects may depend upon such regional differences. The hypothalamus was selected for examination since its serotonin content is known to be among the highest of mammalian brain areas and since its content of, and suspected regulation by, neurohumoral substances is of great interest. The epiphysis cerebri or pineal organ, a probably glandular derivative of the central nervous system, has the greatest concentration/weight of serotonin(6) and has a distinctive serotonin metabolism, leading via hydroxyindole-O-methyl transferase activity to formation of melatonin (7,8) and possibly other compounds.

Methods and materials. Four-week-old, Long-Evans, male rats, born and raised in the same suite of windowless, artificially lighted (14 hours/day) rooms were used in replicate experimental series in which siblings were distributed between their original litter cage with standard laboratory chow and solid floor and sides (= cage controls) and individual suspended wire mesh cages (Bussey type) containing a powdered, standard, controlled diet mixture without amino acid supplement (= diet controls), or the same diet containing 7% L-phenylalanine, or the same diet containing instead 5% L-tryptophan. The standard diet was 20% ground gluten, 76% ground whole yellow maize, 3% calcium carbonate, and 1% sodium chloride. Complete vitamin supplement, including viosterol, was mixed with this. Animals were weighed at the start and at termination of the experiments and were fed *ad libitum.*

All animals were sacrificed by decapitation during the mid afternoon of the daily light period because this is the period of relatively high level and relatively greatest stability in the daily rhythm in pineal serotonin content(9). A daily rhythm in rats' hypothalamic serotonin content does not seem to occur(9). Animals of diverse categories of age, diets and lengths of treatment were sacrificed in rough alternation. The hypothalamus was dissected out by means of 5 cuts with a razor blade through: first, transverse planes at the posterior edge of the optic chiasma, and the anterior limit of the mammillary nuclei; then, lateral planes extending dorso-laterally toward the medial edges of the caudate nuclei (cauda) from the ventral furrow on the lateral edges of the

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