2. Animals of group 2 grew at a significantly slower rate and were less active than those of group 1.

3. At 26 weeks old group 1 animals had an average of 1·8 carious teeth per rat; in group 2 the corresponding figure was 0·5. This difference is highly significant.

4. Even on the sucrose diet the incidence and severity of the observed dental caries was less than that reported by other workers.

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On the role of corticoids in conditioning the gastric mucosa to certain toxic actions of ergocalciferol

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It was evident from the first systematic studies on the pathological histology of ergocalciferol intoxication that the stomach is particularly often and severely affected by overdosage with this vitamin. Rats treated with heavy doses of Vigantol (an irradiated ergosterol preparation kindly supplied by Merck and Co. Inc.) regularly exhibited three distinct layers of calcium deposition in the mucosa, submucosa and muscularis of the stomach (Selye, 1929).

These observations have assumed renewed interest for us more recently, when we found that certain types of pathological calcification depended largely upon the 'conditioning action' of adrenocortical hormones. Rats receiving a solution of 1% (w/v) $NaH_2PO_4.H_2O$ or anhydrous Na_2HPO_4 in water as a drinking fluid tended to develop widespread calcification in the zona intermedia of the kidney if they were

simultaneously given deoxycorticosterone acetate (DOC-Ac), whereas cortisol* acetate (COL-Ac) exerted an opposite effect, in that it inhibited this type of nephrocalcinosis (Selye & Bois, 1956*a–c*; Selye, Bois & Ventura, 1956). An ever-growing number of observations shows, furthermore, that corticoids can condition (sensitize or desensitize) a great variety of tissues to the potentially pathogenic actions of various agents. In this manner the cortical hormones are often the decisive factors determining susceptibility to disease (for literature cf. Selye & Heuser, 1955–6).

It is the object of this communication to report experiments that show the calcification of the gastric mucosa occurring under the influence of ergocalciferol to be likewise largely dependent upon the actions of certain corticoids.

EXPERIMENTAL

Eighty female Sprague–Dawley rats, having an average initial body-weight of 100 g (range 93–110 g), were divided into four equal groups and all were injected daily subcutaneously with ergocalciferol (1 mg in 0·2 ml. sesame oil). One group received no further treatment, a second group had a daily subcutaneous injection of COL-Ac (1 mg of microcrystals suspended in 0·2 ml. water), a third group of DOC-Ac (1 mg of microcrystals suspended in 0·2 ml. water) and the fourth group had both, as indicated in Table 1. Throughout the experiment the animals of all groups were fed exclusively on Purina Fox Chow (a well-balanced mixture prepared for laboratory rats by Ralston Purina Co. Ltd), containing 1·86% calcium and 1·31% phosphorous, and were given ordinary tap water as drinking fluid.

All animals were killed with chloroform after 10 days of treatment. Half of each stomach was fixed in neutral 10% formalin (for the subsequent staining of calcium deposits with Kossa's silver-nitrate stain), the other half in Susa solution (for the demonstration of mucin with the Hotchkiss–McManus PAS stain). Sections 6 μ thick were cut at intervals of about 1 mm throughout the stomach. These Kossa-stained sections were scored for the severity of calcification on an arbitrary scale of 0 to + + +, and the total score was divided by the number of rats in each group to obtain the mean degrees of gastric calcification. In ten animals of each group the aorta, heart, kidneys, one lung and adrenals were also fixed in formalin for the detection of any calcium deposits outside the stomach.

RESULTS

Our results are summarized in Table 1. In group 1 ergocalciferol alone did not produce any calcium deposition in the stomach, except for occasional traces in two animals. However, the same amount of ergocalciferol given in conjunction with COL-Ac produced marked calcification in the gastric mucosas of all animals of group 2. In the rats of group 3, which were treated conjointly with DOC-Ac and ergocalciferol, the gastric mucosa was free of calcium deposits, except for traces in three animals. The rats of group 4, which received treatment with both COL-Ac and DOC-Ac as well as with ergocalciferol, showed appreciably less calcification than those of group 2.

* Also widely known as hydrocortisone.

The sections gave the impression that DOC-Ac in fact counteracted the sensitizing effect of COL-Ac. Since we had twenty animals in each group this inhibition was significant ($P<0{\cdot}01$), although we believe such histological observations do not readily lend themselves to statistical analysis. On the other hand, there can be no doubt about the fact that COL-Ac given alone or along with DOC-Ac conditioned the gastric mucosa to the calcifying action of ergocalciferol (Pl. 1, 1,2).

Table 1. *Effect of ergocalciferol on calcification of the gastric mucosa as influenced by corticoids*

(Mean values after 10 days' treatment. All rats received 1 mg ergocalciferol daily)

Group no.	No. of rats	Treatment with corticoids	Degree of calcification in stomach*
1	20	None	0 or trace
2	20	COL-Ac, 1 mg daily	$1{\cdot}9\pm0{\cdot}19$
3	20	DOC-Ac, 1 mg daily	0 or trace
4	20	COL-Ac, and DOC-Ac, 1 mg of each daily	$0{\cdot}8\pm0{\cdot}23$

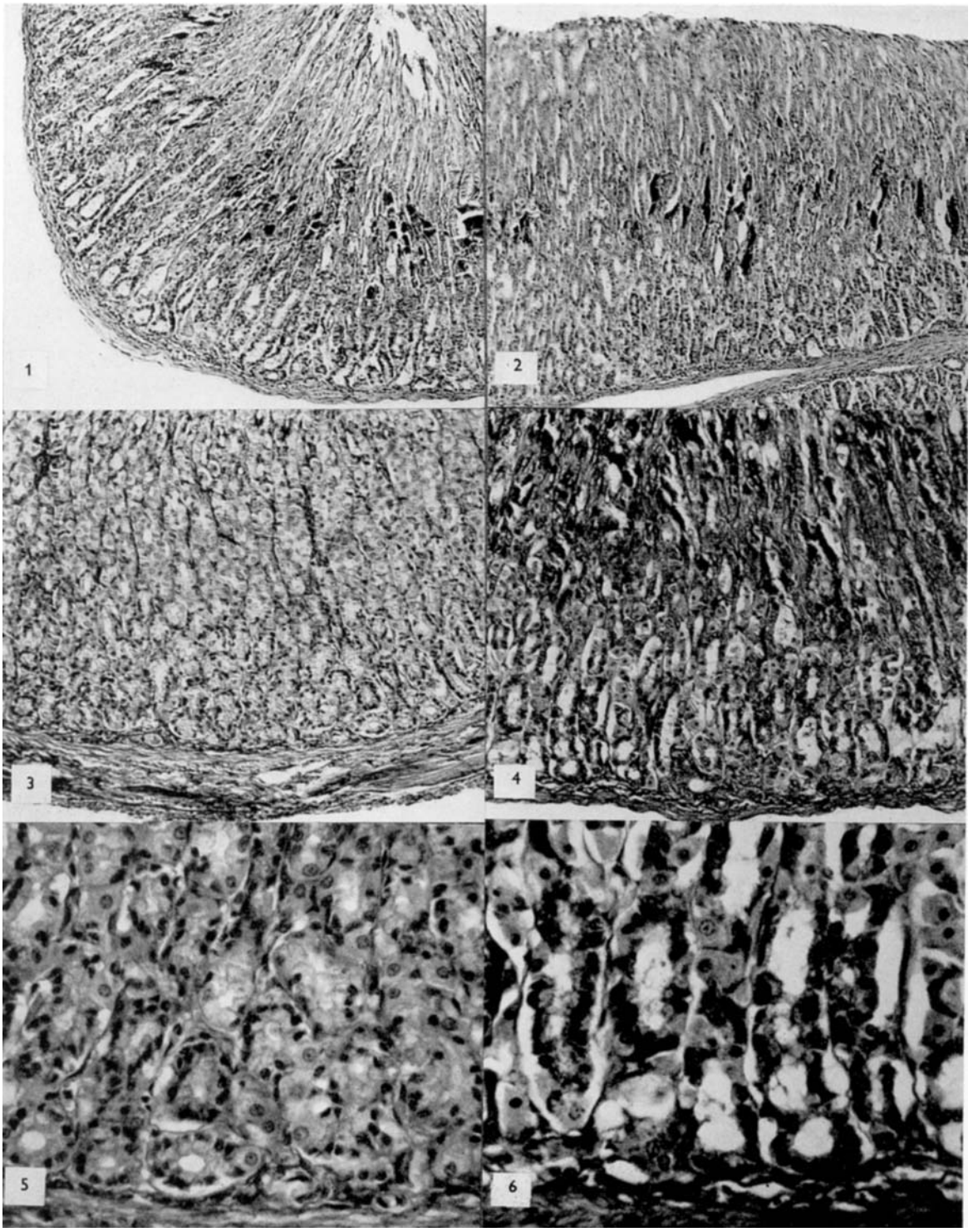
* Scored on an arbitrary scale from 0 to +++. $P<0{\cdot}01$ in the comparison of groups 2 and 4.

It is also noteworthy that, in all the animals of groups 2 and 4, in which calcium depositions had been obtained by treatment with both ergocalciferol and COL-Ac (alone or with DOC-Ac), there was an excessive secretion of PAS-positive gastric mucus, which, together with the calcium deposits, tended to obliterate the lumens of the gastric glands, so that their basal portions were greatly dilated with retained secretion (Pl. 1, 3–6).

DISCUSSION

The results show that a dose of ergocalciferol that, under ordinary conditions, produces no obvious change in the gastric mucosa of otherwise untreated rats elicited marked calcification in animals simultaneously treated with a glucocorticoid hormone, such as COL-Ac. These calcium deposits were extracellular and located in the stroma of the gastric mucosa. This is yet another instance of a morbid change whose development is largely dependent upon the adrenal cortex. In this instance, we can speak of a truly selective conditioning of one target organ, since our previous observations have demonstrated that calcification caused by other agents and in other sites—for instance, the nephrocalcinosis induced by excess dietary phosphate or by mercurial intoxication —is inhibited by COL-Ac treatment (Selye & Bois, 1956*a–d*).

The excessive mucin formation and the dilatation of the gastric glands under the influence of treatment with ergocalciferol and COL-Ac together are perhaps only the secondary consequences of irritation and obstruction of the gastric glands by calcium deposits. However, DOC-Ac, which notoriously counteracts many of the characteristic actions of COL-Ac (for instance, its anti-inflammatory effect), also appears to inhibit sensitization by COL-Ac to pathological calcium deposition in the stomach. Under our experimental conditions, this inhibition was incomplete and perhaps not truly significant, but there can be no doubt that DOC-Ac did not share the sensitizing effect



(*Facing p.* 21)

of COL-Ac on calcium deposition in the stomach. This point appears of interest, because the experimental nephrocalcinosis produced in various ways is markedly aggravated by DOC-Ac (Selye & Bois, 1956*a–d*).

Under the experimental conditions here described, calcium deposition in the stomach occurred selectively in the middle layer of the mucosa, and there were no detectable calcium deposits in other soft tissues of the body. We mention this because more intense and long-lasting overdosage with ergocalciferol produced three distinct layers of calcium deposition in the mucosa, the submucosa and the muscularis, as well as in many other organs, such as the heart, kidneys, lung, adrenals and aorta (Selye, 1929).

SUMMARY

1. Eighty female Sprague–Dawley rats of average initial body-weight of 100 g were divided into four equal groups and given daily subcutaneous injections of ergocalciferol. Group 1 was used as control. Group 2 received additional daily subcutaneous injections of cortisol acetate, group 3 of deoxycorticosterone acetate, and group 4 of both. Throughout the experiment all animals were fed on Purina Fox Chow.

2. It was observed in these experimental animals that subcutaneous injections of ergocalciferol, which in itself causes no pathological calcifications, induced calcium deposition in the gastric mucosa if given simultaneously with cortisol acetate.

3. Deoxycorticosterone acetate did not share this sensitizing effect of cortisol acetate; indeed, it appeared to inhibit it.

4. It is concluded that the adrenocortical steroids exerted a selective 'conditioning' effect upon the ability of ergocalciferol to induce pathological changes in the gastric mucosa of the rat.

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EXPLANATION OF PLATE

1 and 2. Two widely separated regions from the gastric mucosa of a rat treated with ergocalciferol and COL-Ac. The fairly sharp delimitation of the black, silver-stained calcium deposits in the middle part of the gastric glands throughout the stomach should be noted. Stained with Kossa's silver-nitrate stain. × 65.

3. Gastric mucosa of a control rat treated with ergocalciferol alone. Stained with Hotchkiss–McManus PAS stain. × 100.

4. Gastric mucosa of a rat treated with ergocalciferol and COL-Ac. The accumulation of strongly staining mucus near the surface and the dilatation of the basal part of the gastric glands should be noted. The calcium depositions are not visible with this staining technique, which is meant to bring out mucopolysaccharides. Stained with Hotchkiss–McManus PAS stain. × 100.

5. High magnification of a region near the basal part of the mucosa shown in 2. The glands are normal. Stained with Hotchkiss–McManus PAS stain. × 340.

6. High magnification of a region near the basal part of the mucosa shown in 3. The great dilatation of the glands, which is apparently secondary to obstruction in more superficial segments, should be noted. Stained with Hotchkiss–McManus PAS stain. × 340.

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The nitrogen:water ratios of albino rats and their use in protein-evaluation tests

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In the search for shorter methods for protein evaluation the nitrogen:water ratios of the animals commonly used in these tests have recently become important. Bender & Miller (1953*b*) found that in their black-and-white hooded rats the N:H_2O ratio was of such constancy that it could be used for calculating carcass nitrogen from a knowledge of the water content and the age of the rat. This easy way of estimating carcass nitrogen is an important time-saving factor in the carcass-nitrogen method of protein evaluation proposed by Bender & Miller (1953*a*). These authors, however, doubted whether their calculated regression equation for N:H_2O values, correlated with the age in days, would also be applicable to other rat colonies (Bender & Miller, 1953*b*) and the existence of strain differences was indeed observed later by Forbes & Yohe (1955) when a small number of albino rats was analysed. These authors also concluded that significant errors (up to 20%) can be introduced into determinations of net protein value when the carcass nitrogen content is obtained by relying upon the predetermined N/H_2O × 100 constants—a conclusion that suggests variation in these constants even when the rats are of similar age.

The object of this paper is to present on a larger scale further findings on the stability of the relationship between the N:H_2O ratio and age of our albino rat colony and to report on an observed difference between the N:H_2O ratios of male and female animals.