the ability of formaldehyde to fix cells without appreciably changing their nature.

The final trial again indicated the association of T_3-I^{131} uptake with percentage of motile spermatozoa in a given sample. All treatments exhibited the greatest decline in T_3-I^{131} uptake between day 0 and 2, which probably reflects, to a certain extent, the degree of cold shock suffered by the cells in their respective treatments. The spermatozoa suspended in N-J solution cooled to 7°C and undoubtedly cold-shocked by this treatment showed the greatest decline. Apparently, the spermatozoa on this treatment also already showed the effects of cold shock at the time of the first assay, as their uptake value was relatively low even though the degree of motility exhibited was similar to that of the other treatments. Thus, in this instance, the uptake of T_3-I^{131} was more indicative of the effects of cold shock than motility. At the second assay, day 2, both measures reflected the effects of cold shock on the sperm cells.

Summary. The uptake of triiodothyronine-I^{131} by washed bull spermatozoa was found to have a positive association with sperm cell concentration and period of incubation with the labeled T_3. Spermatozoa suspended in a simple salt solution (2.9% sodium citrate) had a higher uptake of T_3-I^{131} than spermatozoa diluted in a more complex ionic medium (N-J solution). The T_3-I^{131} uptake by the sperm cells suspended in the 2 diluents increased at different rates with an increase in cell concentration. Within the range studied, addition of glucose to the 2 diluents had no effect on uptake of T_3-I^{131} by spermatozoa. Sperm cell viability greatly influenced their uptake of T_3-I^{131}. Cells killed by continuous incubation at 38°C or by treatment with acetic acid or sodium hydroxide had similar isotope uptake values which were significantly less than that of live cells. Spermatozoa killed by cold shock treatment, however, had the lowest uptake of T_3-I^{131}. Whereas, formaldehyde treatment of spermatozoa resulted in uptake values significantly greater than that of live cells. A positive association between uptake of T_3-I^{131} and motility occurred with spermatozoa stored up to 18 days in different diluents and temperatures.

1. Flipse, R. J., J. Dairy Sci., 1954, v37, 425.
2. ———, ibid., 1956, v39, 923.
3. ———, ibid., 1960, v43, 881.
4. Crawford, M. D., Flipse, R. J., Almquist, J. O., ibid., 1956, v39, 522.
5. O'Dell, W. T., Almquist, J. O., Flipse, R. J., ibid., 1959, v42, 89.
6. Iype, P. T., Abraham, K. A., Bhargava, P. M., J. Reprod. Fertil., 1963, v5, 151.
7. Sullivan, J. J., Mixner, J. P., Proc. Soc. Exp. Biol. and Med., 1962, v109, 974.
8. Norman, C., Johnson, C. E., Porterfield, I. D., Goldberg, E., Dunbar, R. S., Jr., J. Dairy Sci., 1961, v44, 1180.
9. Wales, R. G., Wallace, J. C., J. Reprod. Fertil., 1964, v8, 361.

Received November 12, 1964. P.S.E.B.M., 1965, v119.

Influence of Exogenous Calcium on Calciphylaxis and Calcergy.* (30088)

G. GABBIANI† AND B. TUCHWEBER (Introduced by H. Selye)
Institut de Médecine et de Chirurgie expérimentales Université de Montréal, Montreal, Canada

Previous studies carried out to test the calcifying potency of various substances when placed in contact with the tissues of living animals have suggested the following classification: 1) *Calcergens,* which elicit calcification in unpretreated animals(1,2,3), 2) *Calciphylactic challengers,* which induce calcification only in animals sensitized by substances capable of increasing the calcium and phosphate content of blood and inter-

* This work was supported by Lakeside Laboratories, Inc. and the Medical Research Council of Canada.

† Fellow of Medical Research Council of Canada.

TABLE I. Comparative Effects of Ca-Ac Upon Calcergy and Calciphylaxis.

Group	Treatment*	Calcification			Blood level immediately before lead, Fe-Dex and PMX injections (mg/100 ml)	
		Heart (scale 0-3)	Kidney (scale 0-3)	Skin (mm) PMX injection site	Ca	P
1	Pb-Ac	0	0	28 ± 4.4	10.51 ± .24	9.92 ± .22
2	Pb-Ac+Ca-Ac	0	0	0	12.14 ± .30	6.92 ± .15
3	DHT+Fe-Dex	0	.1	19 ± 2.9	12.29 ± .38	13.10 ± .28
4	DHT+Fe-Dex+Ca-Ac	.6	2.8	38 ± 1.7	14.81 ± .24	9.73 ± .20

* In addition, all rats received PMX 30 μg in 0.2 ml water s.c.

stitial fluids (*e.g.*, vitamin-D derivatives or parathyroid extract)(1).

We do not yet know the mechanism by which calcium salts are precipitated *in vivo* but there exists a relationship between the calcifying potency of various substances and their solubility in Tyrode solution(4): compounds which induce calcification in normal animals (calcergens) precipitate phosphate or carbonate from the usual Tyrode solution, whereas compounds which induce calcification in animals pretreated with vit. D (calciphylactic challengers) precipitate phosphate or carbonate only from Tyrode solution adjusted to have approximately the same electrolyte content as the serum of vit. D pretreated animals.

Subsequent experiments have demonstrated that various calcergens, administered intravenously, predispose for the production of connective-tissue calcification at the site of subcutaneous phosphate injection(5), and that calcergy cannot be initiated in the presence of low blood phosphate even when associated with a high calcium level(6). These tests suggest that phosphate plays the leading role in the mechanism of calcergy.

On the other hand, there are indications that calciphylaxis may behave differently. Parathyroid extract, which is a very potent calciphylactic sensitizer can, if administered at the proper time, completely inhibit calcergy(6). The availability of calcium is essential for production of calciphylaxis(7,8), and this ion seems to be principally involved in the development of this reaction(9).

In the experiment to be described here, we have compared the effect of oral calcium administration on calcergy and calciphylaxis.

Methods. Eighty female Sprague-Dawley rats of the Holtzman strain, with a mean initial body weight of 100 g (range 95-110 g) were subdivided into 4 equal groups and received the following treatment, as indicated in Table I.

Dihydrotachysterol, or DHT (Calcimin®, Dr. A. Wander, S. A., Bern, Switzerland) 1 mg in 0.5 ml corn oil by stomach tube once on the first day.

Ferric Dextran, or Fe-Dex (Imferon®, Benger Laboratories, Holmes Chapel, England) equivalent to 1 mg of iron in 1 ml of water intravenously once on the second day.

Lead acetate, or Pb-Ac [$Pb(C_2H_3O_2)_2 \cdot 3H_2O$, Fisher Scientific Co., Fair Lawn, N. J.] 3 mg in 1 ml water intravenously once on the second day.

Calcium acetate, or Ca-Ac (Fisher Scientific Co.) ½ mM in 2 ml of water orally 24 hours, 18 hours, 1 hour and 30 minutes before the Pb-Ac and Fe-Dex injections.

Polymyxin, or PMX (Polymyxin-B sulfate, Pfizer Co., Montreal, Canada) 30 μg in 0.2 ml of water subcutaneously immediately after the Pb-Ac and Fe-Dex injections.

Ten rats of each group were sacrificed just before administration of lead, Fe-Dex and PMX, and blood samples were taken to determine the influence of oral administration of Ca-Ac on blood calcium and phosphorus levels of normal and DHT treated animals. Blood calcium and phosphorus levels were analyzed after the Clark and Collip(10), and Fiske and SubbaRow techniques(11) respectively. The mean values with their standard errors are listed in Table I.

Throughout the experiment the rats were maintained exclusively on Purina Laboratory Chow (Purina Co. of Canada) and tap water. The experiment was terminated on the fifth day by killing the animals with chloroform.

At autopsy, the diameter of the calcified

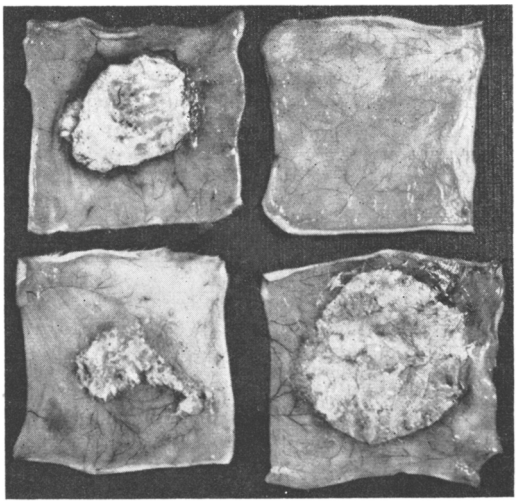

FIG. 1. *Prevention of calcergy and aggravation of calciphylaxis by Ca-Ac. Top left:* Calcergic lesion induced by Pb-Ac and PMX. *Top right:* Complete prevention by Ca-Ac administration. *Bottom left:* Calciphylactic lesion following DHT, Fe-Dex, and PMX treatment. *Bottom right:* Aggravation by Ca-Ac.

wheals produced by topical treatment was measured in millimeters and the means with standard errors are listed in Table I. The intensity of cardiac and renal lesions was gauged in terms of an arbitrary scale of 0 = no lesion, 1 = just detectable, 2 = moderate, 3 = most severe lesions, as described elsewhere(1). The mean intensities of these lesions are listed in Table I. Specimens of the skin and kidneys were fixed in alcohol-formol (4 parts of absolute alcohol and 1 part of 10% neutral formalin) for subsequent embedding in paraffin and staining with the von Kóssa technique for the histochemical demonstration of calcium phosphate and carbonate.

Results. In the animals treated with Pb-Ac and PMX (calcergy) a whitish, brittle plaque of calcification could be seen at the site of PMX injection (Table I). Histologically, mineralization was noted in the connective-tissue fibers of both subcutis and dermis. On the other hand, the rats pretreated with Ca-Ac did not show any calcification macroscopically (Fig. 1) and microscopically the connective tissue also appeared completely normal.

Calciphylactic treatment with DHT, Fe-Dex and PMX resulted in slight renal calcification and cutaneous lesions similar to those of the calcergen-treated animals. Moreover, in the animals pretreated with Ca-Ac, not only were the calcified skin plaques significantly increased in diameter ($P<0.001$) but the renal lesions were also aggravated. Microscopically, the skin lesions appeared similar to those of the calcergen-treated animals. The renal calcification was almost entirely localized in the cortex, particularly in the convoluted part of the proximal tubules; here, the basal membranes were most affected (Fig. 2).

The chemical determinations in the animals killed just before the evocative treatments showed that Ca-Ac administration (Group 2) significantly increased blood calcium ($P<0.001$) while decreasing both blood phosphorus ($P<0.001$) and Ca $\times$ P product ($P<0.001$) in comparison with normal animals. On the other hand, the same Ca-Ac pretreatment in calciphylactically sensitized animals (Group 4) increased blood calcium ($P<0.001$) and decreased both blood phosphorus ($P<0.001$) and Ca $\times$ P product ($P<0.001$) in comparison with DHT treated animals.

Discussion. Oral administration of large amounts of calcium should logically predispose for calcification. Previous work has demonstrated, however, that calcium itself cannot act as a sensitizer for production of calciphylaxis(1,12). The present experiments show that pretreatment with Ca-Ac markedly increases not only the metastatic calcification induced by DHT overdosage(13), but also the very selective cutaneous calciphylactic lesions. It is noteworthy that this aggravation takes place in the presence of a lower Ca $\times$ P product than that of the DHT sensitized animals.

More striking is the prevention of calcergy by the oral pretreatment with calcium. This action may be explained by the fact that calcium administration lowers the blood phosphorus; there is evidence that a normal blood phosphorus level is essential for initiation of calcergy, irrespective of the value of calcium and of the Ca $\times$ P product(6).

In any event, we may conclude that calciphylaxis and calcergy, which in many re-

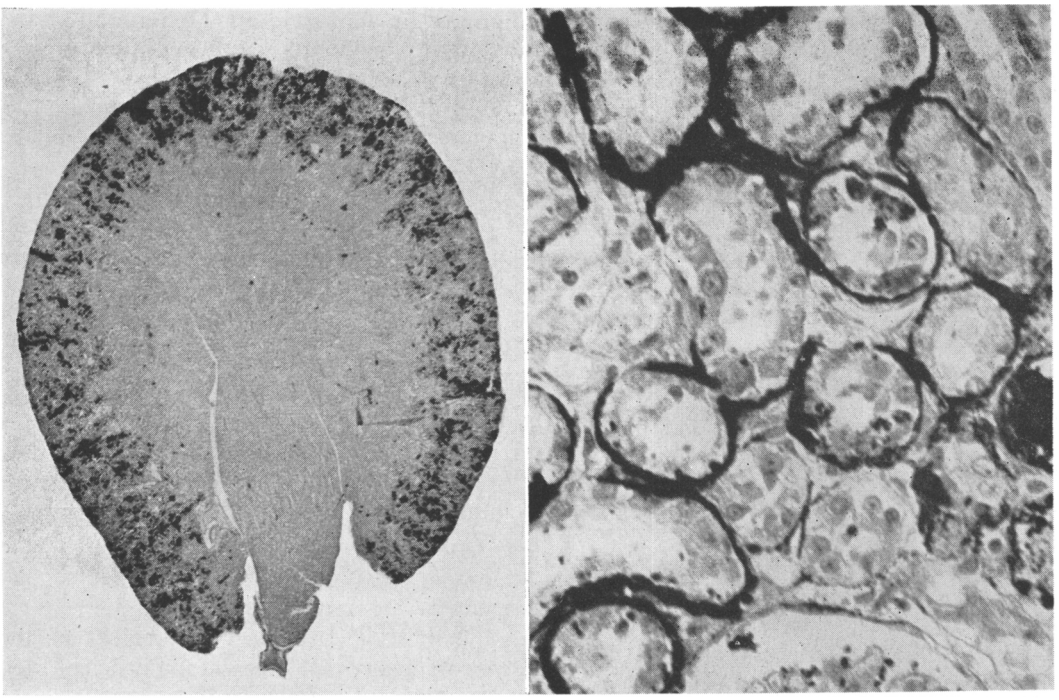

FIG. 2. *Kidney lesions induced by Ca-Ac in calciphylactically sensitized animals. Left:* Calcification is almost completely localized in the cortical region. *Right:* Higher magnification showing localization of calcium salts in basal membrane of tubules (von Kóssa + azure A, × 480).

spects seem related and similar, show a complete difference in their response to oral administration of calcium.

Summary. Experiments on rats have shown that pretreatment with calcium acetate prevents the skin calcification elicited by means of calcergy and aggravates the cutaneous and renal lesions induced by calciphylaxis. This observation suggests a difference in the mechanisms of the two phenomena.

The authors wish to thank Benger Laboratories Ltd. for the supply of Imferon® and Pfizer Co. Ltd. for the supply of Polymyxin-B sulfate.

1. Selye, H., Calciphylaxis, Univ. of Chicago Press, 1962.

2. Selye, H., Gabbiani, G., Fed. Proc., 1964, v23, 339.

3. Şerafimov, N., Tuchweber, B., Gabbiani, G., Jean, P., Selye, H., Arch. int. Pharmacodyn., in press.

4. Gabbiani, G., Experientia (Basel), 1964, v20, 514.

5. Gabbiani, G., Tuchweber, B., Canad. J. Physiol. Pharmacol., 1965, v43, 177.

6. ———, Acta endocr. (Kbh.), in press.

7. Jean, P., Fed. Proc., 1964, v23, 289.

8. Jean, P., Desaulniers, D., Canad. J. Physiol. Pharmacol., 1965, v43, 235.

9. Moss, M. J., Urist, M. R., A.M.A. Arch. Path., 1964, v78, 127.

10. Clark, E. P., Collip, J. B., J. Biol. Chem., 1925, v63, 761.

11. Fiske, C. H., SubbaRow, Y., ibid., 1925, v66, 375.

12. Selye, H., Gabbiani, G., Tuchweber, B., Recent Progr. Hormone Res., 1964, v20, 33.

13. Selye, H., Sr. Adrian Marie O. P., Jean, P., J. Invest. Derm., 1961, v37, 7.

Received November 30, 1964. P.S.E.B.M., 1965, v119.