THE EFFECT OF CORTISONE ON THE REACTION TO QUARTZ IN THE PERITONEAL CAVITY.

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Received for publication October 9, 1951.

CORTISONE is known to affect the rate of healing of wounds by retarding the proliferation of new blood-vessels and by diminishing the amount of collagen formed. The present experiments were designed to determine whether cortisone has a similar inhibitory effect on the fibrosis in chronic inflammation.

Finely powdered quartz was selected as the agent for producing chronic inflammation, as the results could be expected to be more consistent than if a bacterial infection were employed. The quartz was given intraperitoneally, this being a simple and reliable method of producing silicotic fibrosis. By these experiments we hoped to determine not only the possible effects of cortisone on silicosis in particular, but also perhaps to learn something of the fundamental effects of cortisone on the development of granulation tissue in general. The results show that with the dosage used, cortisone suppresses the fibrosis in mice, retards it in rabbits and to a less extent in rats, and has no appreciable effect in guinea-pigs.

METHOD.

Rabbits, guinea-pigs, rats and mice were used, and the procedure of introducing quartz into the peritoneal cavity was based on the technique of Miller, Sayers and Yant (1934).

Powdered quartz (Snowit II prepared from a sand) with a particle size of less than 2μ was made up as a 10 per cent suspension in saline and sterilized by autoclaving. It was then agitated with glass beads in a Kahn shaker for about an hour to break up any aggregations and produce a homogeneous suspension. A single measured dose of this was given intraperitoneally to each animal. In rabbits and rats it was inserted through a laparotomy under ether anaesthesia, the wound subsequently being sutured in layers. In guinea-pigs and mice it was given by simple intraperitoneal injection without anaesthetic.

Half the number of animals of each species were given subcutaneous injections of cortisone acetate daily (Table), commencing on the day the quartz suspension

TABLE.—*Dosage of Cortisone Related to Body Weight.*

	Daily dose of cortisone (mg.).	Average weight (g.).	Dose per kilo (mg.).
Rabbit	20	2500	8
Guinea-pig	10	600	16·5
Rat	5	175	28·5
Mouse	2·5	20	125

was administered. The remainder were used as controls, and given daily injections of corresponding volumes of the aqueous suspending vehicle employed in the particular cortisone preparation used.

Pairs of animals consisting of a cortisone-treated one and a control of the same species were killed at varying intervals, and when an animal died the corresponding animal of the pair was killed. The intention was to extend the experiment to 90 days, but only in the case of the rabbit was this achieved, the other species dying before this period had elapsed.

RESULTS.

Mice.—Following intraperitoneal inoculation of 0·3 ml. of 10 per cent quartz suspension into 16 male mice (weight 15–25 g.) 8 were given 2·5 mg. of cortisone acetate daily and 8 were kept as controls. This dosage was not well tolerated, as all those receiving it were dead within 17 days, the controls meanwhile showing no evidence of illness.

By the fifth day after the quartz injection, the powder in the peritoneal cavity had become clumped. In the control the clumps were firmly adherent to the peritoneal surface, while in the cortisone-treated mouse they were lying free within the sac. By the eleventh day there was an obvious macroscopic connective-tissue reaction around the quartz deposits in the control, but in the cortisone-treated animal nearly all the quartz was still lying free. These differences were still obvious at the seventeenth day. In the control at this stage, the quartz collections were surrounded by distinct fibrous capsules forming discrete nodules. Histologically the nodules were composed of cells, mainly fibroblasts and connective-tissue fibres. On the other hand, in the cortisone-treated mouse at the seventeenth day most of the quartz clumps were still free, while a few small collections which had become adherent to the peritoneal surface were practically acellular and not accompanied by any significant degree of fibrosis or other vital reaction (Fig. 1 and 1*a*).

Rabbits.—The 12 animals used weighed between 2400 and 2900 g. and were each given 2 ml. of the 10 per cent quartz suspension intraperitoneally. Six were given 20 mg. of cortisone daily by subcutaneous injection, while the other six which served as controls received instead a corresponding volume of the aqueous suspending agent. One cortisone-treated and one control animal, closely matched according to weight, were killed 15, 30, 45, 75 and 90 days after giving the quartz. One rabbit died on the seventeenth day.

By the fifteenth day the quartz powder had formed macroscopic aggregations. In the cortisone-treated rabbits most of the aggregations were adherent to the peritoneal surface, but some were free. In the control all aggregations were firmly adherent, and were associated with much more fibrosis than in the cortisone-treated animal. Histologically the difference was qualitative as well as quantitative. In the cortisone-treated rabbit, although there were a moderate number of fibroblasts, they were mainly plump and polygonal and were associated with only little reticulin or collagen formation. In the control, on the other hand, the fibroblasts were elongated and accompanied by the formation of many connective-tissue fibres (Fig. 2 and 2*a*).

After 30 days a peculiar and unexpected change was found within the peritoneal sac of the cortisone-treated rabbit. When the abdomen was opened it appeared to be filled with many pink gelatinous polypi in which were entangled

aggregations of quartz. Histologically this polypoid growth was found to consist, to a very large extent, of thin-walled blood-vessels. There were some mononuclear cells between the vessels but only a small amount of fibrous connective tissue (Fig. 3). The vessels lay in a copious structureless ground substance. The nature of the latter was not determined as it did not stain with toluidine blue, Best's mucicarmine stain or by the periodic acid-Schiff technique.

By the ninetieth day this redundant vascular overgrowth and its ground substance had become replaced by fibrous connective tissue, which was widespread and disorderly and bore no apparent relationship to the few quartz nodules sparsely scattered throughout.

The control animals showed none of these diffuse changes, but only nodular deposits of quartz surrounded by pearly white capsules of dense white fibrous connective tissue.

Histologically, also, even after 90 days there was a distinct difference in the reaction immediately around the quartz. In the cortisone-treated rabbit there was a thin fibrous capsule, the bulk of the quartz remaining acellular. In contrast, in the control animal the nodules were very cellular, and consisted to a large extent of the whorled collagenous fibrous tissue typical of silicotic nodules (Fig. 4 and 4*a*).

The cortisone dosage of 20 mg. daily over a long period did not appear to affect the general health of the rabbits and only one died. The animal that was allowed to survive for 90 days remained apparently healthy for that time and lost only 100 g. in weight, the control rabbit during the same time losing 50 g. There was no obvious macroscopic difference in the rate of healing of the laparotomy wounds between the cortisone-treated rabbits and the controls.

Rats.—Adult male rats of a mixed breed and weighing 160–190 g. were used. Each of 12 animals was given 0·5 ml. 10 per cent quartz suspension through peritoneal incision. Subsequently six were given 5 mg. cortisone acetate daily and six controls received an equivalent volume of the aqueous suspending fluid.

This dosage of cortisone seemed to be near the limit of toleration because several of the rats died during the experiment, the longest survival being 58 days.

EXPLANATION OF PLATES.

FIG. 1.—Cortisone-treated mouse at 13 days. 1*a*: Corresponding control. The quartz nodule from the treated mouse is almost acellular, whilst that from the control is very cellular and overrun by fibroblasts. Haemalum and eosin. × 55.

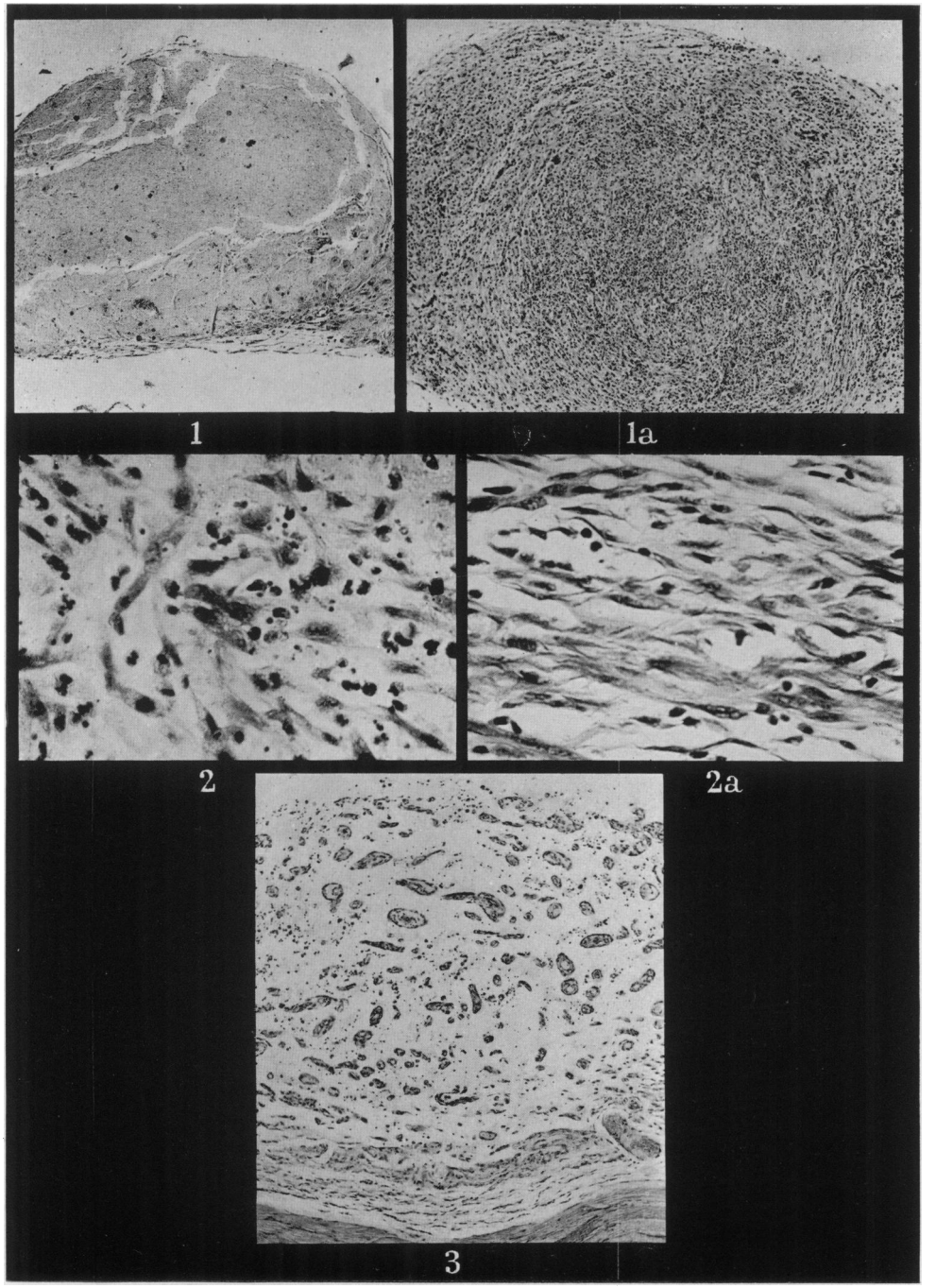
FIG. 2.—From the 15-day cortisone-treated rabbit. 2*a*: Corresponding control. In the treated rabbit the fibroblasts are plump and not associated with much new fibril formation. In the control the fibroblasts are elongated and accompanied by the formation of connective-tissue fibres. Haemalum and eosin. × 360.

FIG. 3.—Abdominal wall of 30-day cortisone-treated rabbit. The polypoid growths consist of many thin-walled blood-vessels in a structureless ground substance. Haemalum and eosin. × 40.

FIG. 4.—Quartz nodule from 90-day cortisone-treated rabbit. 4*a*: Corresponding control. The nodule from the treated rabbit is acellular and surrounded only by a very thin fibrous capsule. In the control the nodule is very cellular and replaced to a large extent by whorled collagenous fibrosis. Haemalum and Van Gieson. × 40.

FIG. 5.—Quartz nodule from a 25-day cortisone-treated rat. 5*a*: Corresponding control. In the treated rat only very few cells and blood-vessels have penetrated into the accumulation of quartz, whereas in the control the accumulation is overrun by cells, especially fibroblasts. Haemalum and eosin. × 60.

FIG. 6 and 6*a*.—Similar sections to Fig. 5 and 5*a*, but stained by silver impregnation. There are many connective-tissue fibres in both nodules, but they are thicker and more numerous in the control. × 60.



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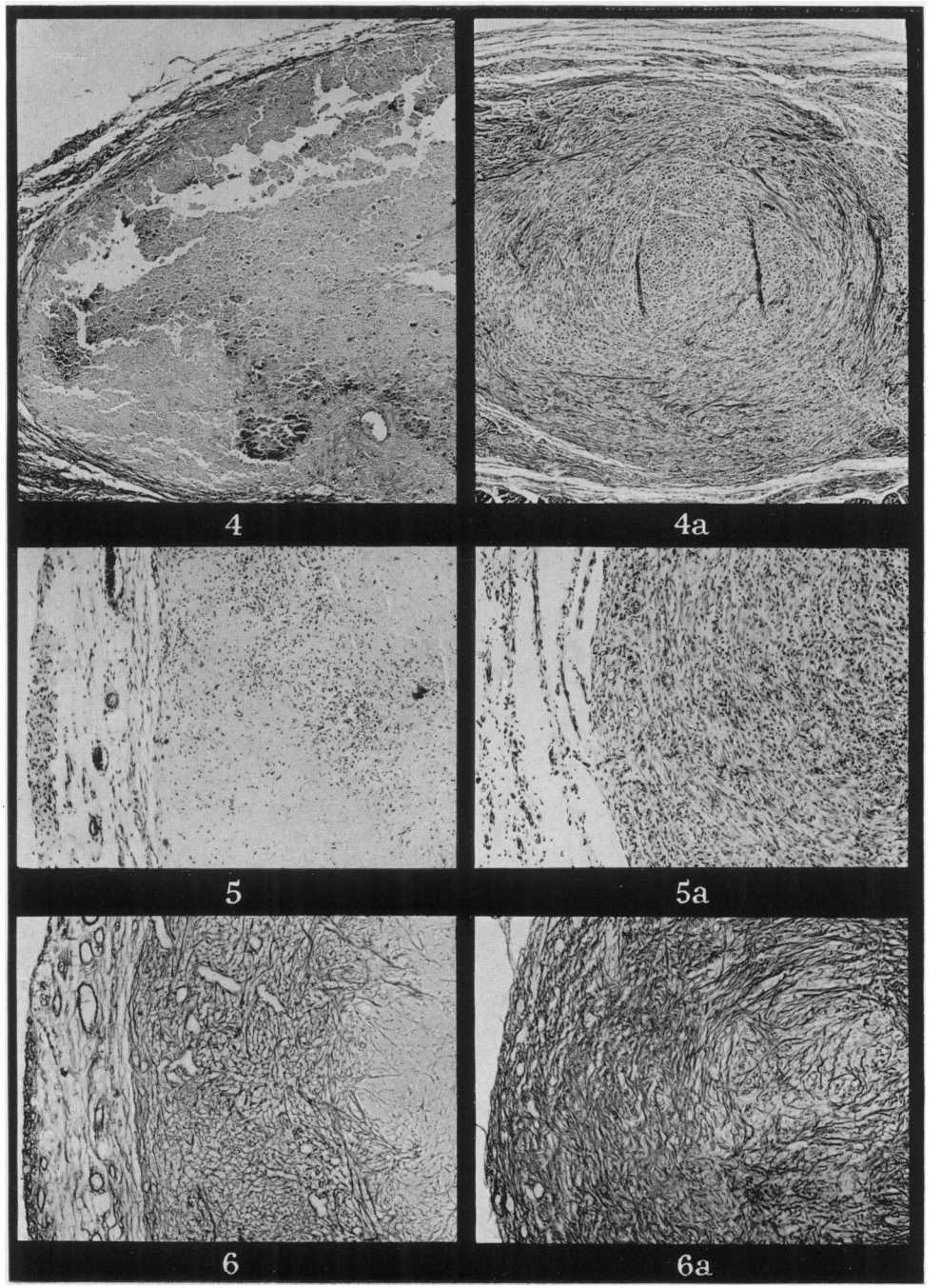

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The control animals meanwhile remained healthy. The cause of death of at least two of the rats was extensive pulmonary aspergillosis.

When the lesions in the cortisone-treated rats were compared with those of the controls, it could be seen that the former showed some diminution and retardation in the formation of fibrous tissue, but the effect was less marked than in the mouse or the rabbit. The difference could nevertheless be well seen after 25 days. When stained with haematoxylin the quartz nodules of the cortisone-treated rats were far less cellular than in the controls (Fig. 5 and 5*a*). With connective-tissue stains, on the other hand, the differences present were less striking ; silver staining, for instance, revealed that fibres had grown into the quartz nodule in the cortisone-treated rat. In the control, however, the new fibres were thicker and more numerous and had overrun almost the whole of the nodule (Fig. 6 and 6*a*).

Guinea-pigs.—Each of 12 male guinea-pigs (weight 480–730 g.) received 1·0 ml. 10 per cent quartz suspension by intraperitoneal injection. Half of this number were given 10 mg. cortisone acetate daily, the other half being controls. Pairs of animals were killed at intervals up to a period of 30 days, the first pair only 5 days after the quartz injection.

At no stage was there any significant difference between the treated and control animals, for in both the quartz powder rapidly formed clumps which adhered to the peritoneal surface and became covered by a firm white fibrous capsule.

DISCUSSION.

The large doses of cortisone used in these experiments suppressed the fibrosis induced by finely powdered quartz in the peritoneal cavity of the mouse, retarded its development in the rabbit and rat and had no effect in the guinea-pig. The relative resistance of the latter species to cortisone has been previously reported. Upton and Coon (1951), who studied the action of cortisone on wound healing, suggested that unlike that in certain other species, connective-tissue formation in the guinea-pig is reduced but little, if at all, by ACTH and cortisone.

Coste, Basset and Delaunay (1951) and Michael and Whorton (1951) have shown that cortisone reduces cellular infiltration in acute inflammation, and several groups of workers have shown that it retards the formation of granulation tissue during the healing of wounds. Nearly all published work has been based on short-term experiments only. Spain, Molomut and Haber (1950) for instance followed the effects of cortisone on wound healing in mice for 5 days. They found very little new capillary formation and only sparse fibroblastic proliferation during that time in treated animals as compared with controls. Ragan, Howes, Plotz, Meyer and Blunt (1949), using rabbits, showed that after wounding, cortisone strikingly depressed new growth of all the connective-tissue elements up to 8 days. Few if any new blood-vessels were to be seen and the fibroblasts lay in a compact arrangement in nests about old blood-vessels, whereas in the controls, fibroblasts were present throughout the wound and fibres had appeared between them. Bangham (1951), also investigating the effect of cortisone on wound healing, carried his observations on for 16 days. He found that the dosage of cortisone he used had no effect in guinea-pigs, that it retarded wound-healing in rabbits and had only a doubtful effect in rats.

Blunt, Plotz, Lattes, Howes, Meyer and Ragan (1950), using experimental fractures in rabbits, found little difference between cortisone-treated animals and controls for the first 3 or 4 days, but beginning with the fourth to fifth days the

differences were striking in so far as there was retardation of all phases of healing, but the period of observation in these experiments was only 8 days. The fibroblasts were noted to have rounded forms with bizarre arrangement of nuclear chromatin, and these features may well correspond with the plump and stellate forms we have found in the rabbit at 15 days. Blunt and his colleagues suggested that the failure of connective-tissue regeneration following fracture in cortisone-treated rabbits may be caused by an inadequate blood supply resulting from the failure of development of new blood vessels.

In our experiments the administration of cortisone was continued much longer, especially in the rabbit, in which it was given for 90 days. Throughout this period there was a great reduction in the amount of fibrous tissue formed in the quartz nodules of the cortisone-treated animals (Fig. 4 and 4*a*). As the object of the experiments was to discover the effects of cortisone on fibrosis over a long period, observations were not made for the first few days, but our findings in no way disagree with those of the above workers who used the shorter term experiments.

In the rabbit an unexpected feature made its appearance by about the thirtieth day. For the first 15 days after the administration of quartz powder there was not a great deal of reaction to its presence in the cortisone-treated rabbit, but in the control animal there was considerable fibrosis immediately surrounding the quartz deposits. By 30 days the peritoneal cavity of the cortisone-treated rabbit was almost filled by pink gelatinous material, which consisted of many thin-walled blood-vessels separated by a copious ground substance. At this stage there was no significant fibrosis in this oedematous-looking tissue. Only later did fibrosis appear, but it was present by 75 days and was diffuse and extensive by 90 days. It appeared to have little relationship to the few deposits of quartz sparsely scattered throughout.

These changes suggest various possibilities. It may be, as suggested by Blunt *et al.*, that cortisone first inhibits vascular proliferation. But in our experiments in the rabbit it would appear that after about 30 days of continuous treatment this inhibition is lost, and the blood-vessels then not only proliferate, but do so in a most disorderly and profuse fashion. This vascular proliferation is soon followed by fibrosis, which being possibly related to and controlled by the growth of the blood-vessels, is itself disorderly and profuse, thus producing the diffuse ragged fibrosis which had developed after 90 days of cortisone treatment.

Another possible explanation of the bizarre changes in the rabbit is that cortisone has an effect on the fibroblasts themselves, as indicated by the work of Barber and Delaunay (1951). The latter showed that the growth of fibroblasts in tissue culture was greatly inhibited by the presence of plasma from animals treated with cortisone. An even greater inhibition was obtained when cortisone itself was added to normal plasma. In our rabbits it may be that cortisone, having delayed the proliferation of fibroblasts and the maturation of collagen, thereby caused an abnormal accumulation of ground substance, and this subsequently became invaded by new blood-vessels and later fibroblasts. We cannot, however, be sure that this is the correct explanation. It may be that the failure to encapsulate the quartz by fibrous tissue allows diffuse irritation as the particles go into solution in the peritoneal fluid.

The initial retardation by cortisone of the development of granulation tissue

is clear from these experiments, but further investigation is needed to determine whether the later redundant fibrosis in the rabbit is also a cortisone effect.

SUMMARY.

Finely powdered quartz was introduced into the peritoneal cavity of mice, rats, rabbits and guinea-pigs and the effect of cortisone on the ensuing fibrosis was studied.

Cortisone inhibited fibrosis in the mouse, depressed it in the rat and had no effect in the guinea-pig.

The findings in the rabbit were anomalous. Fibrosis in the quartz nodules was greatly diminished by cortisone, but following a period of about 30 days there was great vascular proliferation in the peritoneal cavity followed by a disorderly and profuse fibrosis.

We are very grateful to Dr. G. Nagelschmidt for the quartz powder and to Mr. J. P. Napper for technical assistance.

The cortisone used in this work was provided from a generous gift made to the Medical Research Council by Merck & Co., Inc.

REFERENCES.

BANGHAM, A. D.—(1951) *Brit. J. exp. Path.*, **32**, 77.

BARBER, M., AND DELAUNAY, A.—(1951) *Ann. Inst. Pasteur*, **81**, 193.

BLUNT, J. W., PLOTZ, C. M., LATTES, R., HOWES, E. L., MEYER, K., AND RAGAN, C.—(1950) *Proc. Soc. Exp. Biol., N.Y.*, **73**, 678.

COSTE, FL., BASSET, G., AND DELAUNAY, A.—(1951) *Compt. rend. Soc. de Biol.*, **145**, 89.

MICHAEL, M., AND WHORTON, C. M.—(1951) *Proc. Soc. Exp. Biol., N.Y.*, **76**, 754.

MILLER, J. W., SAYERS, R. R., AND YANT, W. P.—(1934) *J. Amer. med. Ass.*, **103**, 907.

RAGAN, C., HOWES, E. L., PLOTZ, C. M., MEYER, K., AND BLUNT, J. W.—(1949) *Proc. Soc. Exp. Biol., N.Y.*, **72**, 718.

SPAIN, D. M., MOLOMUT, N., AND HABER, A.—(1950) *Science*, **112**, 335.

UPTON, A. C., AND COON, W. W.—(1951) *Proc. Soc. Exp. Biol., N.Y.*, **77**, 153.