THE EFFECT OF ANTISERUM, ALONE AND WITH HYDROCORTISONE, ON FOETAL MOUSE BONES IN CULTURE

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Plates 45 to 48

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The experiments to be described were undertaken as part of a programme of research in relation to rheumatoid arthritis, supported by the Nuffield Foundation. Modern work on the pathogenesis of this disease suggests that immunologic reactions, and in particular autoallergy, may be concerned in its causation. As a first stage in our investigations, it was decided to study the direct action of antibodies on bone and cartilage by exposing foetal bones in organ culture to antiserum.

The influence of hydrocortisone on the changes produced in the explants by serum and antiserum was also studied, since previous work has shown that this hormone inhibits the breakdown of cells and intercellular material in culture (1–3) and cortisone is well known to give a temporary remission of symptoms in rheumatoid arthritis.

Material and Methods

Explants.—The explants were obtained from foetal mice near term, the following bones being removed from each foetus: scapulae, humeri, radii, ulnae, femora, and tibiae; in Experiment 462, the scapulae were omitted.

Culture Method.—The culture vessels consisted of round flat-bottomed dishes, 3 cm in diameter and 1.0 cm in height, containing a square table of stainless steel mesh 2.0 mm in height and 2.0 cm in width, to support the explants. Two of these vessels, separated by a siliconed glass rod, were enclosed in a Petri dish 10 cm in diameter carpeted with a ring of absorbent cotton-wool saturated with a 0.9 per cent solution of NaCl. Each culture vessel contained 1.5 ml of medium which just wetted the top of the steel mesh. The Petri dishes were stacked in small vacuum desiccator jars, 3 to 4 dishes to a jar, which were then gassed for 4 minutes with a mixture of air and 5 per cent CO_2, closed, and incubated at 37°C.

At 2-day intervals the medium was sucked off with a Pasteur pipette, the tip of which was bent at an angle, and fresh medium substituted. The explants were then turned over on the grid with a pair of sterile needles. The cultures were maintained for 6 to 8 days after which the explants were fixed for histological study.

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Medium.—

Basic medium: The basic medium used was the protein-free, chemically defined mixture BGJ, devised by Biggers, Gwatkin, and Judah (see reference 4) but supplemented with 5 mg sodium acetate per 100 ml; to this solution, serum or antiserum was added, usually in a concentration of 15 per cent.

Antiserum: Our original antigen preparation was made by passing near-term foetal mice through a mincer of the type designed by Craigie (5), mixing the resultant pulp with an equal volume of Hanks's saline, and freezing and thawing it three times. Rabbits were given intraperitoneal injections of 1 ml of the antigen on 5 successive days followed by 14 days without injections; they then received booster injections on 3 successive days and were bled from ear veins 10 days later. Subsequently when further antiserum was required, the rabbits were given booster doses only. The blood was stored in the refrigerator overnight and the serum separated by centrifugation. Such antisera agglutinated tanned red cells coated with the original antigen at dilutions exceeding 500,000, agglutinated mouse erythrocytes at dilutions of 128, and showed multiple reactions with the original antigen in Ouchterlony plates and on immunoelectrophoresis.

Hydrocortisone: For the experiments with hydrocortisone, hydrocortisone sodium succinate (Solu-Cortef, Upjohn Co., Kalamazoo, Michigan or, for Experiment 462 only, Glaxo Laboratories, Ltd., Greenford, England) was used; it was dissolved in sterile glass-distilled water and added to the culture medium to give the desired concentration, the same amount of water being added to the controls.

Histology.—The bones were fixed for either 30 minutes or 1 hour in Zenker's fluid containing 5 per cent glacial acetic acid followed by either 1 hour or 30 minutes in Zenker's fluid without acetic acid.

Serial sections were cut at a thickness of 7 μ and stained with toluidine blue, with Mayer's acid haemalum, celestine blue and van Gieson's stain, or with Delafield's haematoxylin and chromotrop.

Design of Experiments.—For a direct comparison between the effects of two media, the solutions were placed in two culture vessels in the same Petri dish, and their action on paired rudiments from the same foetus was examined. The design of individual experiments and the number of explants studied are given in table I.

RESULTS

I. Bones at Explantation.—The bones fixed as "zero controls" at the beginning of the experiments (Figs. 1 *a* and 1 *c*) were at an advanced stage of development. The shaft consisted of a fairly thick tube of trabecular bone enclosing haemopoietic tissue; the terminal cartilage at either end comprised a hypertrophic zone which was being excavated and replaced by endochondral bone, a broad proliferative region of flattened cells in the usual columnar array, and an epiphysis in which cell hypertrophy had not yet appeared.

II. Normal Unheated Serum.—Bones were grown in medium containing 15 per cent of unheated serum (18 bones, Experiments 415, 417, and 419) and for comparison others were explanted in BGJ alone (12 bones, Experiments 419, and 423). The explants enlarged considerably in both media, though much less than normal limb bones growing for the same period *in vivo*. There were some important histological differences between the bones of the two series (*cf.* Figs. 1 *b* and 2 *a*). In those grown in BGJ alone (Figs. 1 *b* and 1 *d*), the cartilage re-

tained its metachromasia and the osteoblasts their characteristic appearance; there was very little resorption of cartilage or new ossification, and the marrow cavity was sparsely populated. Explants cultivated in the presence of serum (Figs. 2 *a*, 2 *c*, 4 *a*, and 4 *c*), showed much more cellular activity, both destructive

TABLE I

Summary of Experiments

Exp.	BGJ alone	Normal serum			Antiserum			Normal serum + hydrocortisone			Antiserum + hydrocortisone
		Conc.	Unheated, No. bones	Heated, No. bones	Conc.	Unheated, No. bones	Heated, No. bones	Dose	Unheated, No. bones	Heated, No. bones	Unheated, No. bones
No.		%			%			*µg/ml*			
415		15	6		15	6					
		7.5	6		7.5	6					
		2.0	6		2.0	6					
417		15	6		15	6		7.5	6		6
419	6	15	6	6							
		7.5	6	6							
421					15	4 day culture	4 day culture				
						6	6				
						8 day culture	8 day culture				
						6	6				
423	6				15	6		100.0			6
								10.0			6
								1.0			6
								0.1			6
								0.01			6
429		15		6				100.0		6	
								10.0		6	
								1.0		6	
								0.1		6	
								0.01		6	
439					15	6	6				
445					15	6	6				
462					15	30		10.0			10
								1.0			10
								0.1			10

and formative, than those in BGJ alone. The terminal cartilages were smaller, owing partly to greater resoprtion from the marrow cavity and partly to loss of intercellular material associated with diminished metachromasia; most of the osteoblasts had assumed a fibroblastic form, had multiplied actively and deposited a fibrous "osteoid" material which sometimes filled the marrow cavity of the smaller bones (radius and ulna). There were many osteoclasts and in some explants large areas of bone had been removed and replaced by osteoid tissue.

A concentration of 7.5 per cent of serum (12 bones, Experiments 415, and 419) produced a similar but more drastic effect; on the other hand in 2 per cent of serum (6 bones: Experiment 415) the explants differed little from those in BGJ alone.

III. Unheated Antiserum.—Cultivation in BGJ plus 15 per cent antiserum (72 bones: Experiments 415, 417, 421, 423, 439, 445, and 462) had a disastrous effect on the explants (Figs. 4 *b*, 4 *d*, 4 *e*, 5 *a*, 5 *c*, 7 *a*, and 8 *a*); the degree of damage varied somewhat in different experiments being most severe in Experiment 417, and least in Experiments 439 and 462 which will be described separately.

The muscle and connective tissue surrounding the bone was largely dead by the 8th day, as also were most of the osteocytes and some of the chondrocytes. Recognisable osteoblasts had disappeared, but osteoclasts were abundant and resorption of bone was so extensive that in some of the explants of Experiment 417 (Figs. 4 *d* and 4 *e*) the shaft was reduced to little more than a tube of fibrous periosteum with slug-like osteoclasts (Fig. 4 *e*) sticking to its inner surface. No new bone or fibrous osteoid material was formed. The middle segment of the marrow cavity was sparsely populated by spindle-shaped "fibroblasts" (Figs. 4 *d* and 4 *e*) which were more numerous at the ends of the shaft between the trabeculae of partially excavated cartilage, and more abundant in the radius, ulna, and tibia than in the humerus and femur. The network of endothelial vessels was greatly dilated and in Experiment 417 largely broken down; a variable number of haemopoietic cells, some in mitosis, were usually present. The red blood cells formed large, dense aggregates (Fig. 7 *a*) probably as a result of haemagglutination, and were better preserved than in paired controls in normal serum. The terminal cartilage was smaller in the antiserum and the loss of metachromasia was slightly greater than in the unheated serum (*cf.* Figs. 4 *a* and 4 *b*).

At a concentration of 7.5 per cent (6 bones, Experiment 415), there was much less necrosis than with 15 per cent and the shaft contained many more viable cells. Bone resorption was about as extensive as at the higher level, but the cartilage showed a much greater effect. In explants grown in 2 per cent antiserum (6 bones, Experiment 415) there were few osteoclasts, little bone resorption, and some bone deposition; metachromasia was less than in paired controls in 2 per cent normal unheated serum.

In Experiments 439 and 462 (36 bones) there was very little necrosis except of the muscle, and the cells of the shaft had multiplied vigorously; the marrow cavity was densely populated not only with fibroblasts but also, especially in the smaller bones, with a proportion of typical osteoblasts. Very many osteoclasts were present and active bone resorption was in progress alongside the deposition of plentiful fibrous and osteoid tissue; in many explants the original

bone had almost disappeared. The appearance of the cartilage was similar to that in the other experiments.

IV. The Heat-Inactivation of Serum and Antiserum.—

1. Normal serum (18 bones, Experiments 419 and 429): Heating normal serum to 57°C largely abolished its deleterious effects on the explants (Figs. 2 *b* and 2 *d*). As compared with controls in unheated serum, the bones grown for 8 days in heated serum were longer and had larger terminal cartilages; there was more deposition and very little resorption of bone.

2. Antiserum (24 bones, Experiments 421, 439, and 445): The activity of the antiserum also, was almost if not completely destroyed by heating (Figs. 5 *a* to 5 *d*). There was a spectacular contrast between paired bones in heated and unheated antiserum; explants grown in the former resembled those in heated normal serum, whereas bones in unheated antiserum showed the disastrous changes already described in Section II.

V. The Effect of Hydrocortisone on the Action of Normal Serum and Antiserum.—

1. Hydrocortisone and heat-inactivated normal serum (30 bones, Experiment 429): The effect of hydrocortisone in doses ranging from 100 to 0.01 μg/ml of medium (see Table I) was studied in bones grown for 8 days in heat-inactivated normal serum (15 per cent).

There was surprisingly little difference between the effects of the different levels of hydrocortisone (*cf.* reference 1) and none produced very impressive changes as compared with controls grown in heated serum alone.

The hormone retarded the excavation of cartilage and, except at the lowest dose, both the deposition and resorption of bone.

2. Hydrocortisone and unheated normal serum (6 bones, Experiment 417): There were striking differences between the bones grown in normal serum containing hydrocortisone (7.5 μg/ml) and their paired controls in serum alone (Figs. 3 *a* and 3 *b*). In the former, the cartilaginous ends were much larger, excavation of cartilage and resorption of bone appeared to be arrested and periosteal ossification more active; most of the osteoblasts retained their characteristic appearance.

3. Hydrocortisone and unheated antiserum (66 bones, Experiments 417, 423, and 462): The contrast between bones grown in antiserum with and without hydrocortisone was remarkable (Figs. 6 *a* to 6 *d*). As stated above, the most severe effect of the antiserum was in Experiment 417 (6 bones) in which extensive necrosis coupled with very active osteoclasis, had sometimes reduced the shaft to little more than a tube of fibrous periosteum (Fig. 6 *c*). The addition of 7.5 μg hydrocortisone/ml to this antiserum (Figs. 6 *b* and 6 *d*) abolished the

general necrosis, preserved and encouraged the growth of the terminal cartilage, and almost inhibited bone resorption; most of the osteoblasts retained their normal form and much new osteoid material had been deposited especially on the inner surface of the periosteal bone, where in the radius it was so abundant as nearly to fill the marrow cavity.

In Experiment 423 the effect on the response to unheated antiserum, of concentrations of hydrocortisone ranging from 100 to 0.01 μg/ml, was investigated. In this experiment the anti-serum alone (Figs. 7 *a* and 8 *a*) produced less necrosis than in Experiment 417, though there was extensive osteoclasis (Fig. 7 *a*), excavation of cartilage and fibroblastic transformation of osteoblasts. The hormone had some protective action (Figs. 7 *b* and 8 *b*) at all levels even the lowest. The best result was obtained with 1 μg hydrocortisone/ml (Figs. 7 *a* and 7 *b*); at this concentration there were many normal looking osteoblasts, little bone resorption, and fairly active ossification.

In Experiment 462 (6 days in culture; Glaxo hydrocortisone) 0.1 μg hydrocortisone/ml afforded almost complete protection.

DISCUSSION

The results described above show that unheated antiserum to extracts of whole foetal mice had a drastic effect on foetal mouse bones in culture. These effects could be prevented by heating the antiserum to 57°C for 45 minutes, indicating that the action was complement-dependent. Recently Coombs and Fell (data unpublished) have observed a similar resorption of cartilage and bone matrix in embryonic chick bones grown in the presence of anti-Forssman antiserum, an effect that is completely complement-dependent.

It is interesting that unheated normal rabbit serum produced similar though much less pronounced changes in the bones. The fact that the serum could be inactivated by heat would accord with the view that its deleterious effects may have been due to natural antibodies. The occurrence of such cytotoxic antibodies in normal serum has been demonstrated in cell cultures (6) and to detoxicate sera by heat is common practice in tissue culture (7).

In certain respects the effect of antiserum and to a lesser degree of normal serum, resembled that of excess of vitamin A (8, 9). Recent work by members of the Strangeways Research Laboratory (10–14) has shown that vitamin A causes the breakdown of cartilage matrix by releasing an acid protease from cytoplasmic granules known as lysosomes (15). It seems probable that the lysosomal enzymes are also concerned in the more complex process of bone resorption, though this has yet to be proved.

Since the effects of unheated antiserum and vitamin A on bones in organ culture are so similar, it was natural to wonder whether the antiserum, like the vitamin, activated the lysosomes of the skeletal cells. Bitensky (16) has demonstrated some activation of the lysosomes of ascites tumour cells exposed

to antibody even in the absence of complement, though the response was much greater when complement was present. Weiss and Dingle (3) have shown that, unlike vitamin A (12), antiserum does not release the lysosomal hydrolases by a direct action on the isolated organelles of rat liver, but, when intact rat fibroblasts in culture are exposed to antiserum, acid phosphatase is immediately liberated from the great majority of cells; recently Weiss (data in preparation) has found that even a sublethal dose of antiserum causes loss of acid phosphatase from the fibroblasts. These results support Bitensky's suggestion (16), that antiserum affects the lysosomes indirectly by increasing the permeability of the cell membrane.

This sublethal type of enzyme release may be of greater pathological interest than that which follows cell death. The drastic changes that occur in cartilage and bone exposed to unheated antiserum in organ culture, are probably due to such a "weeping" lesion at the cellular level. It is conceivable that a similar mechanism may be concerned in the chronic rheumatic lesions well known to histopathologists (17).

Cortisone has long been known to have a pronounced antiinflammatory reaction *in vivo* (for a review of earlier work see Dougherty, reference 18). Fell and Thomas (1) found that hydrocortisone greatly retards the dissolution of late foetal mouse bones exposed to excess of vitamin A in culture, and it also reduces the breakdown of cells and intercellular material in explants of foetal rat skin irradiated by ultraviolet light (2). Weissmann thought it possible that in certain pathological conditions in man some of the damage to the tissue might be caused by the release of lysosomal hydrolases from the injured cells, and that the beneficial effect of hydrocortisone on these conditions, might be due to a stabilising action on the lysosomes. This idea received some support from the observation of de Duve *et al.* (19) that cortisone delays the thermal release of enzymes from isolated lysosomes, and by Weissmann and Dingle's finding (20) that lysosomes isolated from the livers of hydrocortisone-treated rabbits were more resistant to ultraviolet irradiation *in vitro*, than were those obtained from normal animals. Recently Weissmann, Thomas, and their collaborators have shown that in other conditions found to cause release of lysosomal enzymes into the blood, *e.g.* experimental shock (21, 22) and endotoxin (21), this release can be partly inhibited by cortisone.

At the cellular level, Rosenau and Moon (23) made the interesting observation that hydrocortisone partially inhibited the cytolytic activity of sensitised lymphocytes of BALB/c mice on homologous cells of the L strain in culture. Recently Weiss and Dingle (3) found that the rapid release of acid phosphatase in fibroblasts exposed to unheated antiserum was completely prevented by the previous or simultaneous addition of hydrocortisone, even when the cells were killed by the antiserum. Weiss (24) has shown also that the rate of uptake of vital dye by cells in culture is retarded by hydrocortisone. These findings indi-

cate that the hormone stabilises not only the lysosomal membranes as postulated by Weissmann, but also the cell membrane. It is probable that the protective action of hydrocortisone on cells and tissues exposed to unheated antiserum in culture, is merely another manifestation of the hormone's general antilytic activity, and is not due to interference with the antibody-antigen reaction itself.

Thus we tentatively interpret the present results to mean (*a*) that unheated antiserum causes a release of lysosomal enzymes with consequent breakdown of intercellular material, (*b*) that this release is due to an indirect action on the lysosomes via an increased permeability of the cell membrane and (c) that hydrocortisone inhibits this effect by stabilising both the lysosomal and cell membranes. It must be emphasized, however, that at present this interpretation of our findings is merely a working hypothesis.

SUMMARY

1. The effects of normal rabbit serum and of rabbit antiserum to whole foetal mouse tissues, on the isolated limb bones of late foetal mice were studied in organ culture, and the influence of hydrocortisone on these effects was investigated.

2. Unheated normal serum caused slight loss of metachromatic material from the cartilage matrix, and some resorption of both cartilage and bone.

3. In unheated antiserum to foetal mouse tissues, the terminal cartilage was smaller and less metachromatic than in paired controls in normal serum, while osteoclasis was so intense that in many explants the bone had almost disappeared. The amount of necrosis varied with different batches of antiserum.

4. The changes produced by normal serum and antiserum could be largely prevented by heating the sera to 57°C for 45 minutes.

5. The effects could also be inhibited by the addition of hydrocortisone to the unheated sera; as little as 0.1 μg hydrocortisone per ml of medium had a well marked protective action.

6. It is suggested that (*a*) unheated antiserum causes a release of lysosomal enzymes with consequent breakdown of intercellular material, (*b*) this release is due to an indirect action on the lysosome *via* an increased permeability of the cell membrane, (*c*) hydrocortisone does not affect the antigen-antibody reaction, but inhibits the autolytic changes that normally follow this reaction, possibly by stabilising both the lysosomal and cell membranes.

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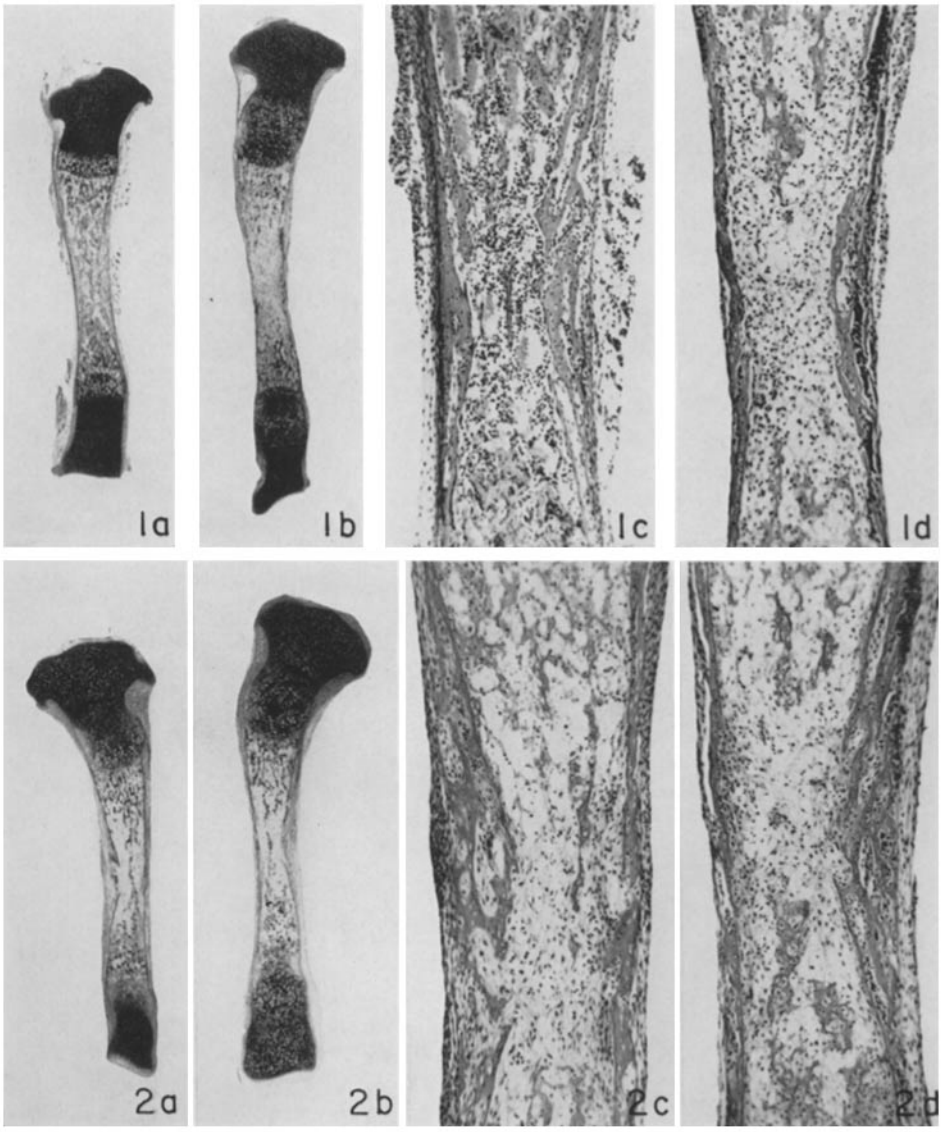
EXPLANATION OF PLATES

The photographs were made by Mr. M. F. Applin; the plates were prepared by Mr. V. C. Norfield.

PLATE 45

FIGS. 1 *a* to 1 *d*. (Experiment 423) Fig. 1 *a* Normal tibia (zero control) from a foetal mouse near term, showing the structure of the rudiment at the time of explantation. Fig. 1 *b* Tibia from a litter mate after 8 days' cultivation in a chemically defined, protein-free medium (BGJ); note enlargement of explant and further differentiation of hypertrophic cartilage. Fig. 1 *c* Another section of Fig. 1 *a* showing the diaphysial bone and marrow. Fig. 1 *d* Another section of Fig. 1 *b* showing bone, slightly reduced in thickness, and marrow from which most of the haemopoietic tissue has gone. (Fig. 1 *a* and 1 *b*. Sections stained with toluidine blue; Figs. 1 *c* and 1 *d*.: stained with Mayer's acid haemalum, celestine blue, and Van Gieson. Figs. 1 *a* and 1 *b*, × 14. Figs. 1 *c* and 1 *d*, × 59).

FIGS. 2 *a* to 2 *d*. (Experiment 419) Fig. 2 *a* Tibia grown for 8 days in BGJ plus 15 per cent normal unheated rabbit serum. Note greater resorption of cartilage than in BGJ alone (*cf.* Fig. 1 *b*) and some loss of metachromasia from the distal end. Fig. 2 *b* Opposite tibia from the same foetus as Fig. 2 *a*, after 8 days' growth in BGJ plus heated normal rabbit serum; the explant is larger than in Fig. 2 *a* and there is less resorption of cartilage and loss of metachromasia. Fig. 2 *c* Another section of Fig. 2 *a*, showing bone and marrow cavity. Fig. 2 *d* Another section of Fig. 2 *b*, showing bone (staining and magnifications as in Fig. 1).

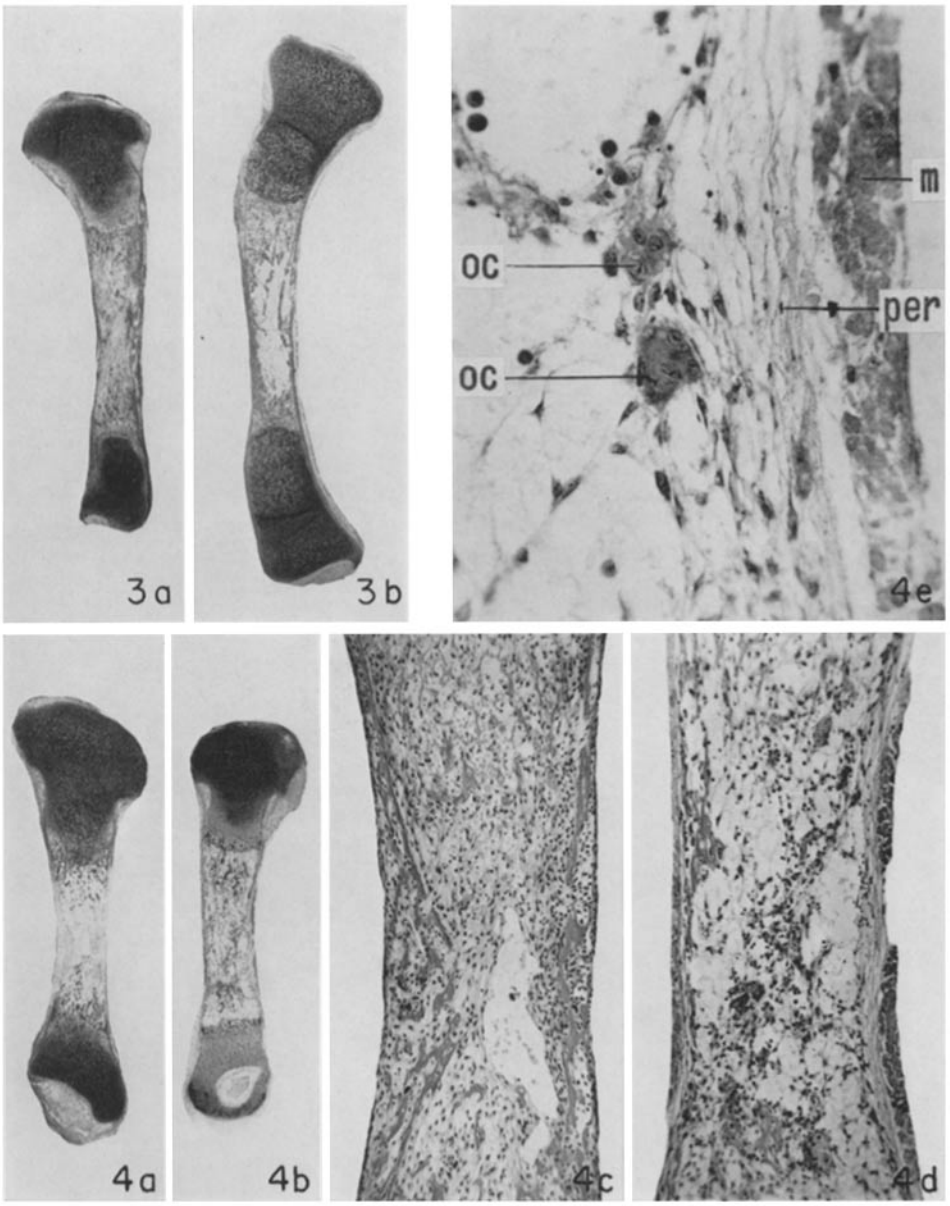


(Fell and Weiss: Effect of antiserum on foetal mouse bones)

PLATE 46

FIG. 3 *a* and 3 *b*. (Experiment 417). Fig. 3 *a* Tibia grown for 8 days in BGJ plus unheated normal rabbit serum; note resorption of cartilage and partial loss of metachromasia (*cf.* Fig. 2 *a*). Fig. 3 *b* Opposite tibia from the same mouse grown in the same medium as Fig. 3 *a* but with the addition of 7.5 μg hydrocortisone (HC)/ml of medium; the rudiment is larger than Fig. 3 *a* and the resorption of cartilage is greatly reduced. (Stain, Delafield's haematoxylin and chromatrop. × 14).

FIGS. 4 *a* to 4 *e*. (Experiment 417). Fig. 4 *a* Femur grown for 8 days in BGJ plus normal unheated rabbit serum. Fig. 4 *b* Femur from a litter mate grown in BGJ plus unheated antiserum to whole foetal mouse tissues; note extensive shrinkage and resorption of cartilage as compared with Fig. 4 *a* and loss of metachromasia (complete disappearance at the proximal end). Fig. 4 *c* Another section of Fig. 4 *a* showing diaphysial bone which is somewhat rarefied and marrow cavity largely occupied by fibroblast-like cells and endothelial vessels. Fig. 4 *d* Another section of Fig. 4 *b*; the bone has disappeared completely on the right side of the shaft leaving only the fibrous periosteum, and is greatly reduced on the opposite side; note sparse population of cells in the marrow cavity and large clear spaces, apparently caused by dilation and breakdown of endothelial vessels. Fig. 4 *e* Another section of Fig. 4 *b* showing osteoclasts (*oc*) and remains of periosteum (*per*); the bone has completely disappeared in this area; *m*, muscle. (Stain: Figs. 4 *a*, and 4 *b*, toluidine blue; Figs. 4 *c*, to 4 *e*: Mayer's acid haemalum, celestine blue, and Van Gieson. Figs. 4 *a* and 4 *b*, × 14. Figs. 4 *c* and 4 *d*, × 59. Fig. 4 *e*, × 350).

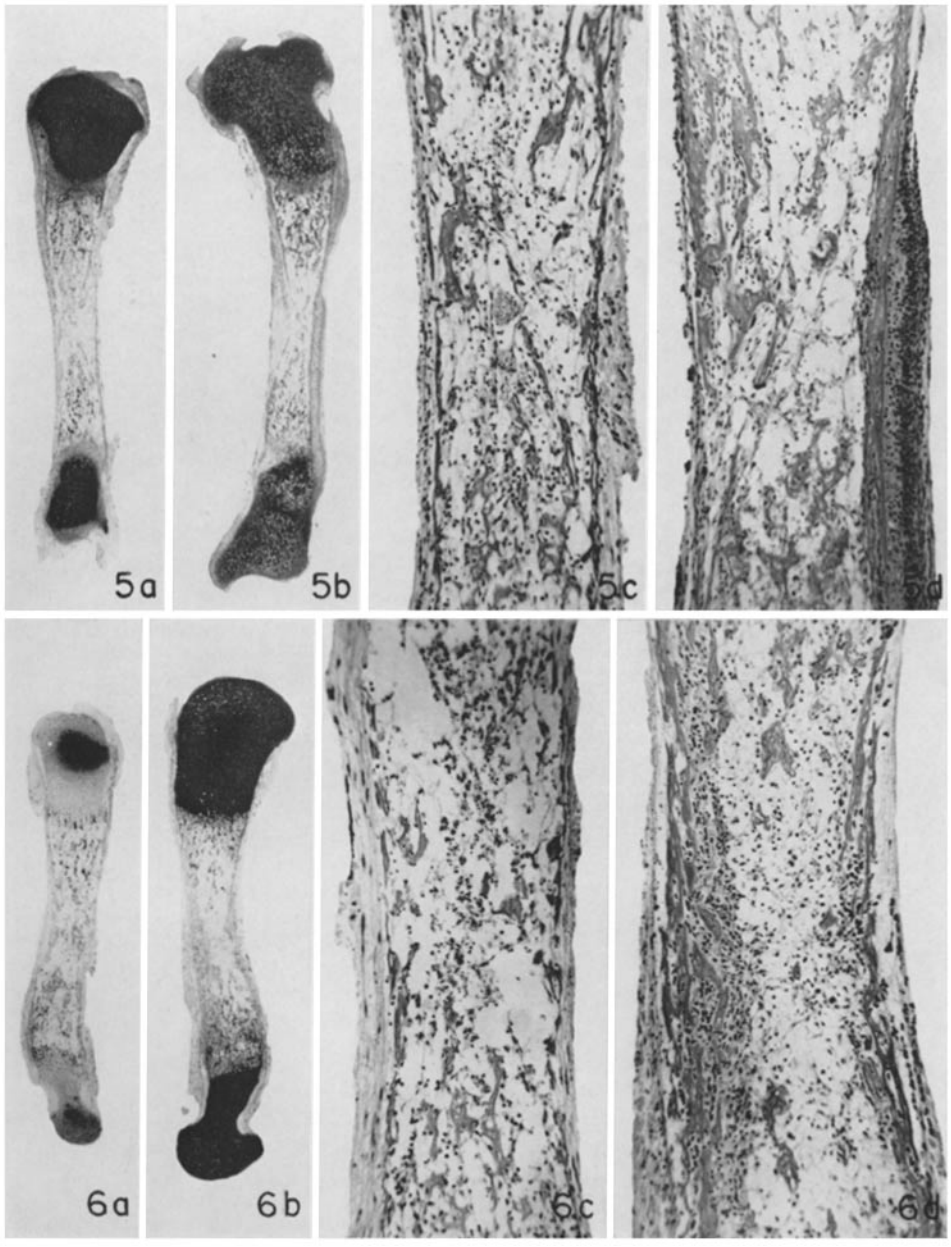


(Fell and Weiss: Effect of antiserum on foetal mouse bones)

PLATE 47

FIGS. 5 *a* to 5 *d*. (Experiment 421). Fig. 5 *a*. Tibia grown for 8 days in BGJ plus unheated antiserum to foetal mouse tissues; the explant is similar to that shown in Fig. 4 *b*, but rather less drastically affected. Fig. 5 *b* Opposite tibia from the same foetus grown in BGJ plus heat-inactivated antiserum; the effect of the antiserum has been almost abolished. Fig. 5 *c* Another section of Fig. 5 *a* showing extensive bone resorption. Fig. 5 *d* Another section of Fig. 5 *b*; note well preserved bone and new periosteal ossification on the right of the shaft. (Stain: Figs. 5 *a* and 5 *b*, toluidine blue; Figs. 5 *c* and 5 *d*, Mayer's acid haemalum, celestine blue, and Van Gieson. Figs. 5 *a* and 5 *b*, × 14. Figs. 5 *c* and 5 *d*, × 59).

FIG. 6 *a* to 6 *d*. (Experiment 417). Fig. 6 *a* Humerus grown for 8 days in BGJ plus unheated antiserum to foetal mouse tissues, showing effects similar to those seen in Fig. 4 *b*. Fig. 6 *b* Opposite humerus from the same foetus, grown in the same medium as Fig. 6 *a* but with the addition of 7.5 μg HC/ml; the effect of the antiserum has been almost completely inhibited. Fig. 6 *c* Another section of Fig. 6 *a*; the bone has almost disappeared from the shaft. Fig. 6 *d* Another section of Fig. 6 *b*; bone resorption and necrosis are greatly reduced (Staining and magnifications as in Fig. 5).



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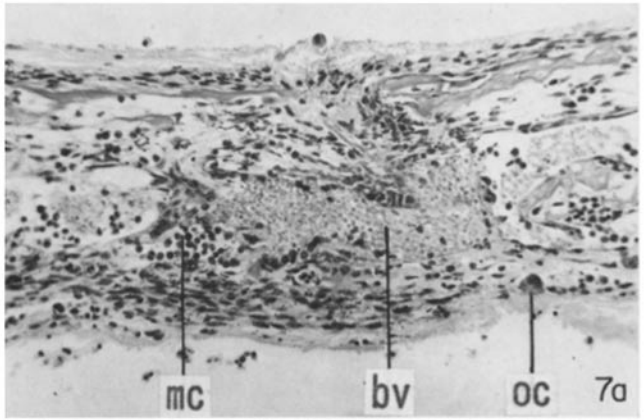
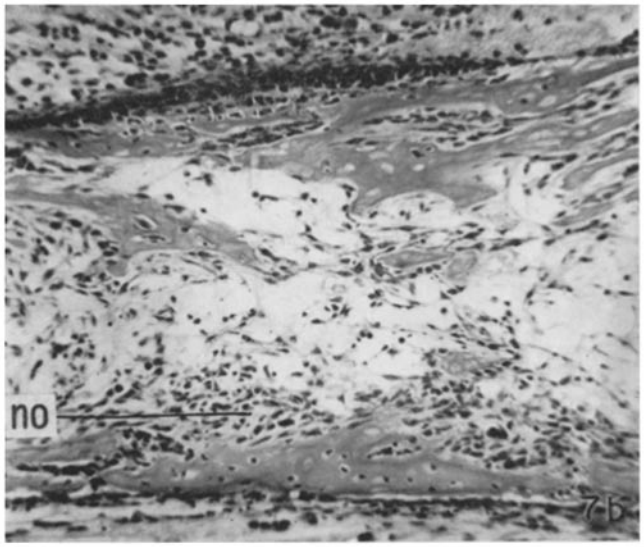
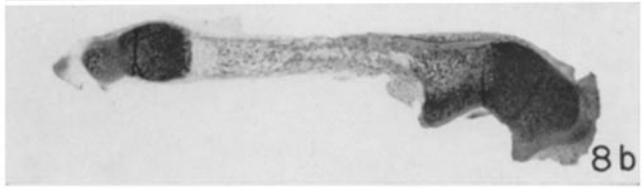
FIGS. 7 *a* and 7 *b*. (Experiment 423). Fig. 7 *a* Shaft of a tibia grown for 8 days in BGJ plus unheated antiserum to foetal mouse tissues. This antiserum produced less severe cytotoxic effects than some other samples, but resorption of bone is nearly complete. Note blood vessel (*bv*) containing a compact mass of red cells, osteoclasts (*oc*) and marrow cells (*mc*). Fig. 7 *b* Shaft of tibia from a litter-mate grown in the same medium as Fig. 7 *a* but with the addition of 1.0 μg Hc/ml; the effect of the antiserum has been almost completely inhibited; note good trabeculae of bone and new ossification (*no*) beneath the periosteum and on the inner surface of the original bone. (Stain: Mayer's acid haemalum, celestine blue, and Van Gieson. × 140).

FIGS. 8 *a* and 8 *b*. (Experiment 423). Fig. 8 *a* Ulna grown for 8 days in BGJ plus unheated antiserum to foetal mouse tissues; note resorption of bone and cartilage and loss of metachromasia from cartilage matrix. Fig. 8 *b* Ulna from a litter mate grown in the same medium with the addition of 0.1 μg HC/ml; even at this low concentration the hormone has had considerable protective action (Stain. Delafield's haematoxylin and chromotrop. × 14).







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