THE OCCURRENCE OF CHANGES RESEMBLING THE INFLAMMATORY IN SKIN INJURED AND INCUBATED AFTER EXCISION*

By JEAN V. COOKE, M.D., DAVID GOLDRING, M.D., and LAWRENCE I. KAHN, M.D.

(*From the Department of Pediatrics, Washington University School of Medicine, and the St. Louis Children's Hospital, St. Louis*)

Plates 30 to 33

(Received for publication, November 15, 1952)

It has long been known that cellular death does not occur with the death of an animal and that certain tissues for example skin and cornea may remain viable for considerable periods as shown by replantation in a living animal. It has also been shown that physiological function may be preserved under certain circumstances in an organ, the heart for instance, by perfusion techniques after removal from the living body. The development of a pathological lesion by cellular activity after separation of tissue from the living body, however, has not been described. In the experiments to be reported here, it was demonstrated that a proliferative cellular reaction like that occurring in inflammation can be artificially produced by injury of the tissue removed after the death of the animal if it is maintained under conditions sufficing to keep the cells alive. The same type of cellular reaction was also demonstrated on human tissue injured after removal at operation. The first observations were made in the course of experiments in which tissue sections were used to determine the viability of cells after incubation and were amplified after the histological change was seen.

Methods

Rabbits were used in the animal experiments, and the skin was the tissue studied. Of healthy adult albino rabbits weighing from 2.5 to 3 kg. the hair of the thorax and abdomen was clipped, and that which remained was removed by the use of a depilatory (2 parts by weight of barium sulfide and 1 part of "dreft") followed by lavage of the area. The animals were killed by the intravenous injection of 20 cc. of sodium pentothal (100 mg.), and after cessation of respiration and heart beat, an area of the cleansed skin was subjected to thermal trauma. This was accomplished by the application for 30 or 60 seconds of a rubber bag through which hot water at a set temperature was circulating, using only sufficient pressure to maintain firm contact. Actually the burned area of the skin included a large portion of that over the thorax and abdomen and the burning was done as a part of other experimental work. The treated area of skin, including the panniculus carnosus, was rapidly excised, rinsed in three changes of warm physiological saline solution to remove blood, and a small piece taken

* Aided by a grant from the Fern Waldman Memorial Fund.

for section as control before incubation. Normal skin at a distance from the treated area was not studied ordinarily since we were interested in a comparison of the traumatized skin before and after incubation. The remainder of the treated area of skin was incubated in Tyrode solution in a water bath at 37°C. Special precautions were taken to prevent bacterial contamination by sterilizing with dry heat the vessel in which the skin was incubated, and keeping it stoppered with sterile cotton, by sterilizing the Tyrode solution by Mandler infiltration, and by the addition of crystalline penicillin to a concentration of 50,000 units with chloramphenicol (chloromycetin) to 125 mg. per 100 cc. of Tyrode solution. Cultures taken during and at the termination of the experiment remained sterile. The Tyrode solution was continuously oxygenated by bubbling oxygen through it, and the temperature of the bath was kept constant by thermostatic controls. In most instances, the incubation was continued for 16 hours, although its length was varied in some cases as noted. At the end of the incubation period, blocks of the skin cut vertically through it were fixed in 10 per cent formalin for 24 hours after which they were imbedded in paraffin and sections stained by hematoxylin and eosin.

In the experiments on human tissue, skin was also employed as available from various surgical operations. In all instances, it was obtained at the operating room as soon as removed by the surgeon. Normal appearing skin was quickly dissected free from underlying subcutaneous fat, and divided into several portions, one piece being saved for control section and the others traumatized by heat by methods described in the experimental protocol. This was done either by firm contact with a rubber bag through which heated water at a set temperature was circulating, or by similar firm contact with a hollow glass tube of 1 cm. outside diameter through which heated water circulated, or by searing a small area momentarily with a hot soldering iron. In some experiments several small portions of excised skin of about 2 by 2 cm. in size were dipped for 30 or 60 seconds in water or Tyrode solution warmed to a stated temperature. The specimens were then placed in Tyrode solution and incubated at 37°C. This was continuously aerated by oxygen bubbling through, which agitated and kept the tissue moving about in the solution. Bacterial contamination was prevented by the addition of penicillin (50,000 units per 100 cc.) with chloramphenicol (125 mg. per 100 cc.). Cultures taken on blood agar at the conclusion of incubation were always sterile. The observations could not have been made without the use of antibiotics during incubation. In our earlier experiments done with only careful aseptic precautions the fluids constantly became contaminated, and the cellular accumulations to be described after incubation were not found.

FINDINGS

In a study of many sections of smooth appearing normal rabbit skin and that taken immediately after thermal injury but before incubation, the corium showed areas in which varying degrees of infiltration with inflammatory cells were seen. These were focal and more frequent immediately beneath the epithelium, although they were also seen around small vessels and hair follicles. The cells were lymphocytes and histiocytes with sometimes a few polymorphonuclear leukocytes. Not all sections showed such infiltration, nor did all areas in the same section. Sections of rabbit skin after incubation showed similar changes which at times appeared more extensive than in normal skin. After examining sections from a number of animals, however, it was difficult to be certain that the changes in the corium after incubation could be attributed to the experimental procedure. In the rabbit skin experiments, therefore, no further reference to possible changes there will be made, and the proliferative changes

in human corium occurring after incubation will alone be described since they are never seen in normal human skin.

In the deeper tissue in and around the panniculus carnosus layer in rabbit skin, cellular infiltration of the sort described was never found in normal animals, and areas of such proliferation seen here after incubation had certainly developed after its removal from the body. They were found immediately beneath the muscle zone, sometimes between the muscle fibers, and in the deeper corium adjacent to the muscle. They consisted of larger or smaller areas of diffuse infiltration by mononuclear cells. Some of these were lymphocytes, but the majority were larger cells with bluish stained protoplasm and a pale somewhat granular nucleus which at times was eccentric. They were indistinguishable morphologically from histiocytes. Some had the morphology of plasma cells. There was only an occasional polymorphonuclear cell. In certain sections, capillaries in the area showed swelling of the lining endothelial cells which were round or oval and resembled the histiocyte-like cells in the adjacent surrounding tissue. No mitoses were seen.

Normal human skin sections never showed any proliferation either in the superficial or deep layers of the corium, and the presence of such changes after injury and incubation was readily demonstrable. Such cellular proliferation was seen in two general locations—(*a*) in the papillary layer of the corium beneath the epithelium where they appeared as focal accumulations often recognizable as being around capillaries, and occasionally scattered diffusely through the tissue; and (*b*) in the deep reticular layer near the subcutaneous tissue where they infiltrated the connective tissue and sometimes that between the fat cells with focal areas of cells in the region of a capillary. The character of the cellular infiltration in the deeper portion of the human skin was apparently identical with that found in the same portion of incubated rabbit skin.

Experiments with Rabbit Skin

Series 1:

The first group consisted of nine rabbits in which the skin of a large area over the thorax and abdomen was burned after death by application of a rubber bag through which water heated to 65°C. was circulating. Sections cut vertically were taken from the skin of each animal immediately after the burning, as controls, and from portions of skin incubated for 16 to 18 hours as described. In the deeper layer of the panniculus carnosus none of the control sections taken before incubation showed any change, but in three of the incubated tissues very definite and rather striking cellular infiltration was seen.

In one specimen the deep tissue beneath the muscle layer showed a striking cellular infiltration in a zone which extended the full length of the section (Fig. 1). Here the connective tissue fibers were somewhat separated by edema, and the cells lay in the widened interstitial spaces. The predominant cell was a well stained mononuclear with moderate amount of slightly basophilic protoplasm, but some cells resembled lymphocytes, and an occasional polymorphonuclear cell was seen. The capillaries immediately beneath the muscle had greatly swollen, round or oval endothelial cells, and around them were small focal cellular accumulations of

mononuclear cells similar to those more diffusely scattered in the loose connective tissue, and lymphocytes (Fig. 2).

In a section from a second animal a similar but more diffuse cellular infiltration was present in the muscle layer itself and between the muscle fibers which were separated by loose cellular connective tissue. A zone of the same cellular connective tissue separated the muscle layer from the outer corium. In addition to the more diffuse cellular change, focal areas of cells were also present in the muscle zone, and at times these were around small blood vessels (Figs. 3 and 4). In certain of the capillaries also the endothelial swelling previously mentioned was apparent.

In sections from a third animal a thin zone of loose connective tissue beneath the muscle layer showed a like diffusely scattered infiltration but to a lesser degree than in the two other specimens described. In skin sections from four other animals no such changes were present, and in two more the voluntary muscle layer was not shown.

The findings described left no doubt that a pathological inflammatory cellular proliferation had occurred in the incubated skin. The predominant cells were of the histiocyte type commonly seen in chronic inflammatory reactions and must have been derived from the fixed tissues. The fact that similar changes were not present in sections from all animals was disappointing. It is possible that although the attempt was made to treat all specimens alike, the conditions may not always have been optimum for cellular survival. Later observations indicated that the degree of thermal trauma to the skin in this experiment may have been excessive.

Series 2:

A second group of six rabbits were treated in a manner identical with the first group described. The control sections taken before incubation showed no abnormality of the deeper corium and panniculus carnosus. Of the specimens of incubated skin, sections from five had diffusely scattered histiocytes in a thin zone of loose connective tissue beneath the muscle layer. The reaction was considerably less than the reactions previously described, but quite definite, and the cells were of the same character. In the sixth specimen the muscle layer was not shown.

In the next experiment an attempt was made to determine the length of incubation necessary for the production of recognizable proliferative change previously described to develop.

Series 3:

The skin of a rabbit was burned and incubated in the same way as in the previous two series, and sections were made in duplicate immediately after the burning (control), and in other portions of skin after incubation for 4, 8, 12, 15, and 24 hours. In the deeper region of the panniculus adiposus, beneath the muscle layer, one or both specimens showed definite, but slight, diffuse cellular infiltration in the 8, 12, 15, and 24 hour specimens, but none in the control and 4 hour sections. This infiltration was of less degree than in some of the previous experiments and was not greater in those tissues with longer incubation.

The only conclusion warranted in the case of the one rabbit studied was that the changes could be detected after 8 hours of incubation.

An experiment was now carried out to determine whether the milder degree of trauma produced by excision of the skin, handling, exposure to air, etc., without additional thermal injury, would be sufficient to induce similar changes in the tissue.

Series 4:

In seven rabbits, normal skin was excised and incubated as previously described, without any other deliberate trauma. Control sections taken immediately after excision were examined, and others after 18 hours' incubation. In none of the control pieces of skin was there any change in the panniculus adiposus, but in the incubated tissues two of the seven specimens showed cellular infiltration. In them a moderately diffuse scattered infiltration of histiocytes in a thin layer of connective tissue immediately beneath the muscle was to be seen. This was quite definite but of less degree than in the sections of burned and incubated skin in the previous experiments.

This observation suggested that relatively mild trauma may be sufficient to initiate proliferative changes of somewhat less degree than those which follow more severe injury.

Since apparently normal rabbit skin showed inconstant and unpredictable cellular accumulations in the papillary zone of the corium, which made reactions during incubation difficult to recognize, it was decided to make similar observations on human skin for the reason that such cellular reactions are not seen in normal skin until after it is traumatized.

Experiments with Human Skin

The characteristic changes in those tissues of human skin in which proliferative lesions were found after incubation will be described briefly, and this description will not be repeated in the individual experiments since the findings always appeared identical although there was some variation in degree. In the corium the infiltration was always focal and occurred around capillaries, often near the epithelial surface (Figs. 5, 6, and 9) although at times they were seen at a somewhat deeper level (Fig. 7). Sometimes similar foci were present in the stroma around hair follicles and glands. No diffuse infiltration away from these focal accumulations was seen. The cells (histiocytes) had well stained round or slightly oval vesicular nuclei and scanty protoplasm but did not resemble lymphocytes. In the deeper tissue adjacent to the subcutaneous fat, cellular infiltration, when present, was occasionally focal and around small vessels, but was often diffuse with scattered cells in the stroma between the fat cells (Figs. 8 and 10), Here the cells were more isolated, and the nuclei were surrounded by a moderately abundant, slightly basophilic protoplasm. They appeared similar to those encountered in the deeper tissue in rabbit skin.

Series 1:

Skin from the thigh of a 75 year old woman upon whom a mid-thigh amputation was done because of diabetic gangrene of foot.

The skin was divided into several pieces, and each piece was burned on the surface for 1 minute at 68°C. by means of applying a rubber bag through which heated water was circulated. In all portions so treated the epithelial surface became separated from the underlying skin. This had not occurred in rabbit skin similarly treated. These pieces, except for a control which was sectioned without being incubated, were then incubated at 37°C. in oxygenated Tyrode solution for varying periods with antibiotic added, and then sectioned. The incubation periods were 3, 8, 13, 19, and 24 hours. All of the heated skin sections appeared normal,—except for loss of the superficial epithelium—and showed no morphological differences from the control sections.

The tentative conclusion from this test, which appeared to be confirmed by later observation, was that the amount of heat applied had injured the skin so much that the cells were no longer capable of showing the proliferative changes. These were seen in later experiments in which less heat was applied to the skin.

Series 2:

Skin was obtained from the thigh of a 60 year old woman who had a high amputation for gangrene of the foot from embolus.

A. The procedure was identical with that of Series 1 except that the skin was burned at 70°C. for 1 minute. Portions were incubated for 8, 12, 22, 45, and 72 hours. All the sections of incubated skin showed no change except a denudation of the epithelium, and no recognizable difference from the section of an unincubated control. As in the preceding experiment, it was felt that the degree of heat applied was excessive and probably produced cellular death.

B. In addition, portions of skin from this patient were incubated without any thermic trauma for the same periods, 8, 12, 22, 45, and 72 hours. Sections from these showed intact epithelium and no change in the control specimen or in those incubated 8 and 12 hours. In the tissues incubated 22, 45, and 72 hours, however, there was striking cellular accumulation, somewhat more marked in the 45 and 72 hour tissues. These changes were focal areas of increase in cells in the outer layer of skin around small vessels and lymphatics. The cells were histiocytes in the main, although some resembled lymphocytes without much protoplasm and with moderately deeply stained nuclei. The changes were identical with those illustrated in later experiments (Figs. 5, 6, and 9).

It was believed that the definite cellular changes found in these sections of skin in which no deliberate trauma was practiced, indicated that the relatively mild trauma of handling the tissue, cooling to room temperature, and exposure to air for the necessary preliminary period before inoculation may cause sufficient injury to produce the reaction. Apparently, the time required for the changes to develop was more than 12 hours in this experiment, although in a later observation (Series 6) similar changes were noted after 18 hours' incubation. In other similar observations (Series 4 and 5) the changes in the papillary zone after 20 hours' incubation were too slight to be considered positive.

Series 3:

Skin from thigh of a 55 year old woman who had a mid-thigh amputation for arteriosclerotic gangrene of foot.

In this experiment, portions of skin were burned in a central zone by firm contact with a hollow glass tube of 1 cm. outside diameter through which heated water at a set temperature

(Table I) was continuously circulating. After the burning, each specimen was incubated 15 hours at 37°C. The temperature and length of application were varied to see if any differences in tissue reactivity could be detected, as indicated.

The area of skin to which heat was applied was 1 cm. in width, and vertical sections were made through the central burned zone with an adjacent 1 cm. on either side. In the tissues in which a reaction was observed, the degree of cellular increase appeared somewhat greater in the central zone than laterally, but there was also a definite cellular reaction around the vessels in the entire section.

The cellular reaction consisted of well marked focal increase in number of cells around the vessels in the papillary area. There was no change in the deeper zone next to the subcutaneous tissue. The cellular changes in this experiment were very definite and striking, though a temperature of 68°C. applied for 1 minute (No. 7) seemed to produce too much injury for the tissue to respond by a reaction, and a temperature of 61°C. for 1 minute (No. 5) also seemed to be

TABLE I

Specimen No.	Time	Temperature	Cellular reaction
	sec.	°C.	
1 (control)	—	—	−
2	30	56	++
3	60	"	++
4	30	61	++
5	60	"	+
6	30	68	++
7	60	"	−

somewhat excessive since the resulting change was definitely less than in those tissues subjected to less thermal injury. The proliferation in the papillary layer in the central zone is illustrated in Figs. 5 and 6 taken from specimen 2 of this series. It will be seen that the cells in the area shown are numerous and more concentrated around smaller vessels.

Series 4:

Skin from the abdominal wall of a colored female 65 years old was obtained at operation for resection of the bowel for carcinoma.

The skin was burned after removal by firm application of a hollow glass rod through which heated water was circulated as in Series 3. Two portions were burned in this way for ½ minute and two others for 1 minute at 60°C. with later incubation for 20 hours at 37°C. Controls of fresh normal skin and unburned normal skin incubated the same way were included. The papillary zone of all incubated specimens showed relatively slight evidence of cellular increase, which was distinctly less than that in the similarly treated specimens described in Series 3. In the deeper portion of the corium, the two pieces of skin burned for ½ minute at 60°C., and one of the two burned for 1 minute at 60°C. showed areas of definite increase of cells around certain of the smaller vessels. An area in the deeper portion of the corium of a specimen burned for 1 minute is shown in Fig. 7.

These results were interpreted as indicating that the degree and duration of heat applied directly to the skin surface in this experiment were sufficient to injure the skin itself very severely, so that it could not react, but that the underlying tissues receiving less thermal trauma retained the ability to show a reaction. In one of the portions subjected to the longest heat (1 minute) even these deeper tissues showed no change.

Series 5:

Skin from breast of a 39 year old woman who had a complete radical operation done for the removal of an adenocarcinoma.

This experiment was done in triplicate and included (*a*) three pieces of normal skin sectioned as controls, (*b*) three pieces of uninjured skin incubated for 16 hours and sectioned, and (*c*) three pieces dipped for 1 minute in sterile Tyrode solution heated to 56°C. for 1 minute and incubated 16 hours.

The control normal untreated skin had no abnormalities on section, and those portions incubated without deliberate injury showed very slight proliferative changes around the vessels in the papillary layer but none in the deeper tissue. In all three of the specimens dipped in heated Tyrode solution, however, there was a very definite increase in cells, a focal increase in histiocytes around certain of the smaller blood vessels of the papillary layer, and in one specimen a similar finding in the deeper layer adjacent to the subcutaneous tissue.

This experiment illustrated the fact that moderate thermic trauma was followed by a greater degree of cellular reaction than that observed after the mild injury resulting from removal, cooling, and handling.

Series 6:

Skin from thigh of a 65 year old white woman after mid-thigh amputation for gangrene of the leg due to an embolus in the femoral artery.

In this experiment, pieces of skin were dipped for ½ minute in hot water, two pieces being exposed to water at 49°C. and two pieces at 56°C. They were incubated in the usual way for 18 hours at 37°C. The findings are given in Table II.

TABLE II

No.		Papillary layer	Reticular layer
1. Normal skin		−	−
2. Normal skin incubated	*a*	+	−
3. " " "	*b*	+	+
4. Burned skin (½ min. at 49°C.) incubated	*a*	+	−
5. " " " " " " "	*b*	+	+
6. Burned skin (½ min. at 56°C.) incubated	*a*	+	−
7. " " " " " " "	*b*	±	−

In all the skin sections marked + there were a number of focal areas around smaller vessels in the papillary layer with definite well marked accumulations of histiocytes like those previously described. In the only two specimens marked as showing changes in the reticular layer, certain areas of the adipose tissue immediately beneath the deepest portion of the reticular layer showed a number of histiocytes in the connective tissue between the fat cells (Fig. 8), as well as a well marked cellular increase around vessels in the papillary layer (Fig. 9).

It was apparent in this experiment, as shown also in Series 2(*B*) that normal skin without deliberate injury may show cellular changes after incubation quite similar to the reaction following mild thermal trauma.

Series 7:

Skin from the upper leg of a 65 year old man obtained after mid-thigh amputation for arteriosclerotic gangrene of the foot.

In this experiment two pieces of skin were seared on the surface momentarily with a hot soldering iron and incubated at 37°C. for 18 hours. Control sections of normal skin and that taken immediately after burning appeared normal. In each of the two pieces of seared and incubated skin no evident change was present in the papillary zone, but definite cellular accumulations were seen in the deeper reticular area. In the adipose tissue adjacent to the reticular layer was a striking focal perivascular cellular increase of histiocytes, and there was also a moderately diffuse infiltration of the same cells in certain areas of the connective tissue between the fat cells (Fig. 10).

This experiment confirmed the impression gained from previous observations that a sufficient degree of heat applied directly to the skin will injure the cells so severely that they do not respond by cellular changes as do tissues injured less severely, but that often in such instances the subcutaneous tissue which is possibly more resistant and certainly less directly exposed to the burn may still show local increase in cells.

DISCUSSION

In the observations reported, it was shown that in rabbit and human skin after removal from the body there developed a remarkable increase in histiocytes, especially around the smaller vessels of the papillary zone and also in the subcutaneous tissues. Such changes were somewhat more marked after slight thermal injury to the excised tissue than after the milder insult produced by excision, cooling, handling, and exposure to air. They developed only after incubation at body temperature in sterile oxygenated media and could be recognized after 8 to 12 hours. Severe thermal injury inhibited the cellular activity and prevented the development of the characteristic change. There was some variation in the degree of the reaction, but under optimum conditions the changes developed in a considerable proportion of the experiments.

The abnormal cells present were predominantly mononuclear cells having the morphology of histiocytes, although some lymphocytes were also seen and occasionally cells having the morphology of plasma cells. The characteristic location was around capillaries, either in the corium or in the subcutaneous tissue, but at times the cells were also scattered diffusely in the connective tissue and between fat cells. Sometimes in rabbit skin sections they were present in large numbers, especially in the deeper exposed layers of the subcutaneous tissue adjacent to the skin, and often in the papillary zone of human skin the focal perivascular cellular accumulations were abundant, with a moderate scattering of them in the surrounding tissue.

The pathological picture, so far as concerned the cells under consideration, resembled that seen in chronic inflammatory reaction with infiltration of tissue by histiocytes and perivascular cellular reaction. When such perivascular accumulations of cells are seen in inflammatory lesions in living tissue the assumption is often made that they have been mobilized from other points and carried to the involved area by the bloodstream. This is true in the case of polymorphonuclear cells in acute inflammation, but other cells more commonly present in such perivascular infiltrations of subacute and chronic inflammation may not always have a hematogenous origin. The presence of well marked, focal, perivascular accumulations in the excised tissues studied, which were without blood supply, left little doubt that they arose from the fixed tissues since it seems quite unlikely that the cellular accumulations resulted from proliferation of cells present within the capillaries at the time the blood circulation was arrested. This suggests that a similar cellular reaction in chronic inflammatory lesions in the living body may also develop *in situ*.

Since inflammation is usually considered to include the reactions of tissue to injury, it appears likely that the changes observed are in the group of inflammatory lesions, since they resulted from trauma. The lack of circulation prevented the appearance of the usual polymorphonuclear leukocytes, serum, and other substances from the blood, and the response was, therefore, limited to that by fixed tissue elements. It is of interest that the tissue reaction began within a few hours of the injury, like the prompt development of acute inflammatory reactions in the living body, and in timing it was an acute response although the cells involved were those usually associated with chronic inflammation.

Since the accumulations of histiocytes observed were predominantly perivascular, it seemed likely that they arose from a proliferation of the macrophages normally present along the blood vessels (adventitial cells). The motility of such free macrophages during inflammation would explain their presence as resulting from a more or less diffuse infiltration in adjacent tissues. The swollen endothelial cells of the capillaries which were seen in the cellular areas have also been noted in acute inflammatory reactions in living tissue and are probbably distinct from the macrophage proliferation. As previously mentioned, it is unlikely that macrophages of hematogenous origin were concerned in the inflammatory reaction in excised incubated skin thus considered, although hematogenous macrophages are usually believed to play a prominent part in inflammatory reactions in the living body.

SUMMARY

Observations are reported on the changes which occurred in excised rabbit and human skin after mild trauma and incubation at body temperature. These changes resembled those of chronic inflammation, in that perivascular and

diffuse infiltration by histiocytes occurred in the corium and subcutaneous tissue, but they developed within a few hours. The experiments have shown that even after removal from the body certain tissue elements may retain the ability to react with proliferative changes in response to tissue injury if kept under artificially simulated physiological conditions. The possible significance of these changes in relation to the inflammatory process is discussed.

We are indebted to Dr. Zola Cooper, Department of Pathology, Washington University, for reviewing the microscopic sections and confirming the morphological changes as described.

EXPLANATION OF PLATES

PLATE 30

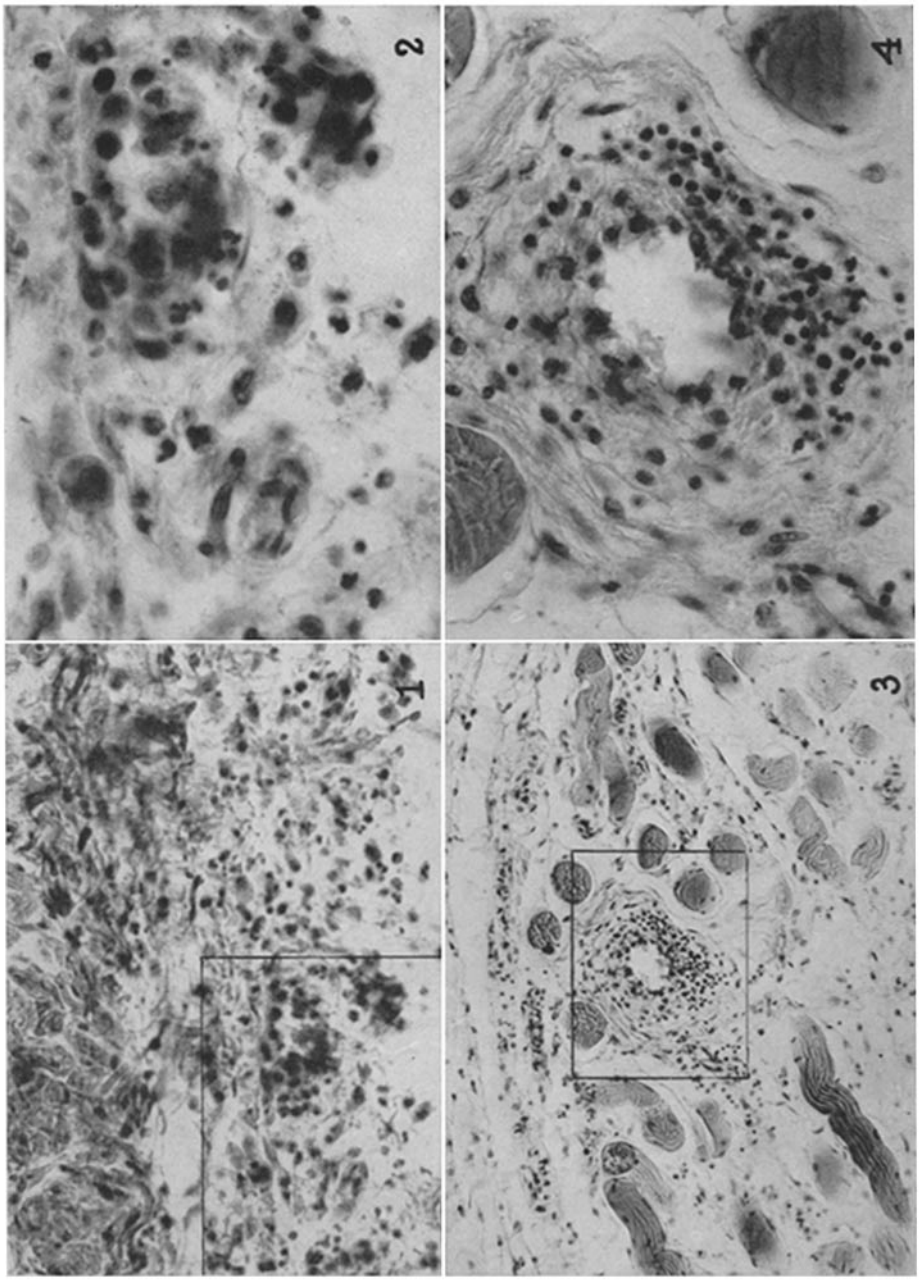
All sections stained with hematoxylin and eosin.

FIG. 1. Rabbit skin. Portion of zone of infiltration by histiocytes beneath voluntary muscle layer attached to corium. × 160.

FIG. 2. Rabbit skin. Area from Fig. 1. Swelling of capillary endothelium. × 340.

FIG. 3. Rabbit skin. Cellular accumulation within voluntary muscle layer of corium. ×80.

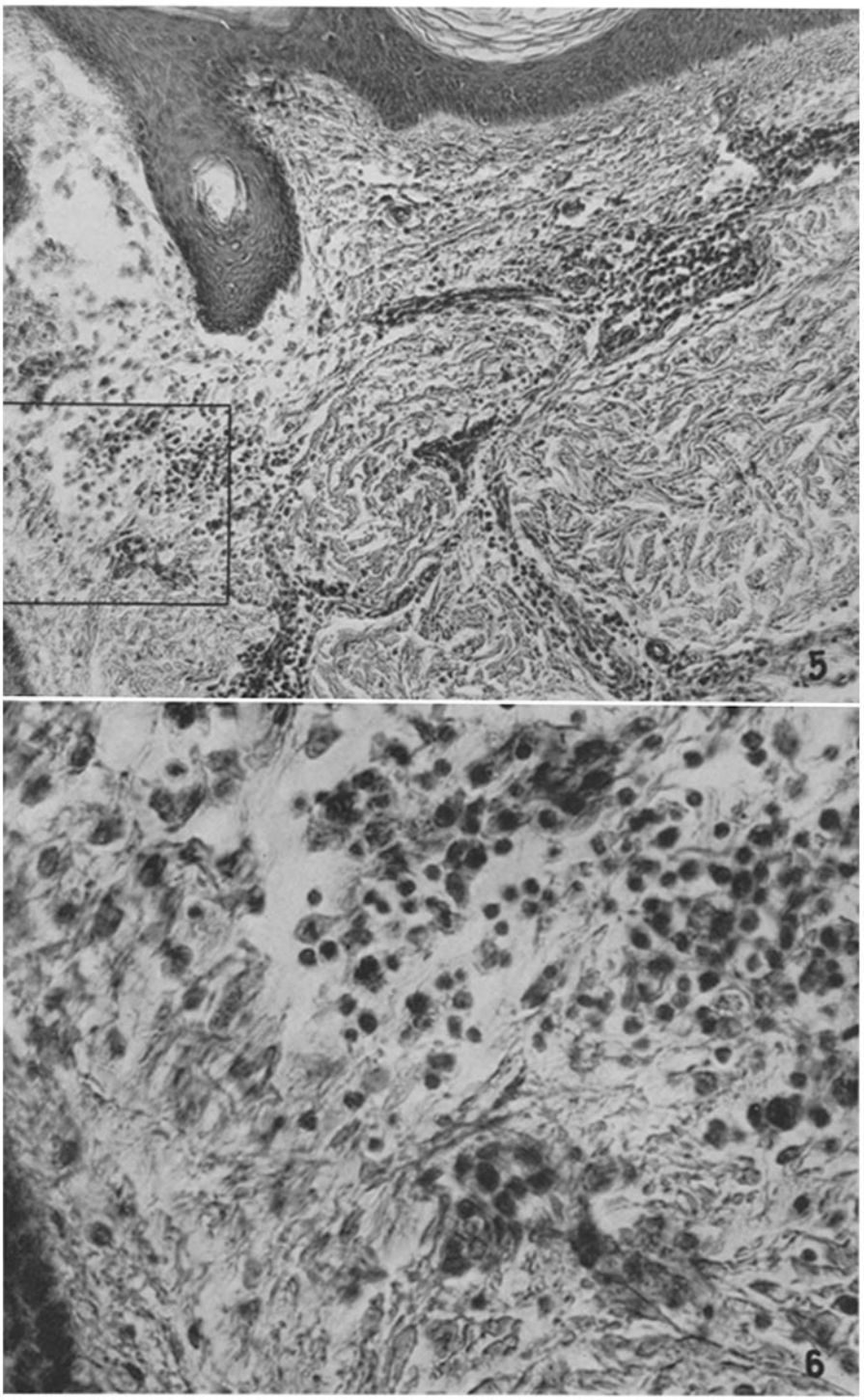
FIG. 4. Rabbit skin. Area from Fig. 1. Vascular endothelial swelling and perivascular cellular accumulation. ×280.



(Cooke *et al.*: Changes resembling the inflammatory in skin)

PLATE 31

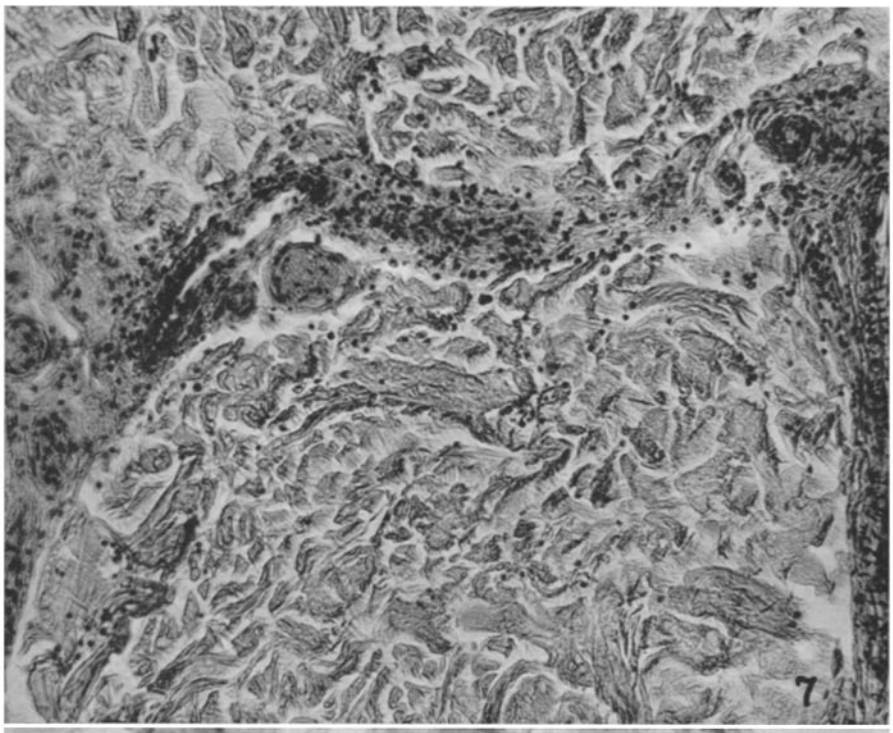
FIG. 5. Human skin. Perivascular cellular increase in papillary zone. × 100.
FIG. 6. Human skin. Area from Fig. 5. Histiocytic increase in papillary zone. × 340.

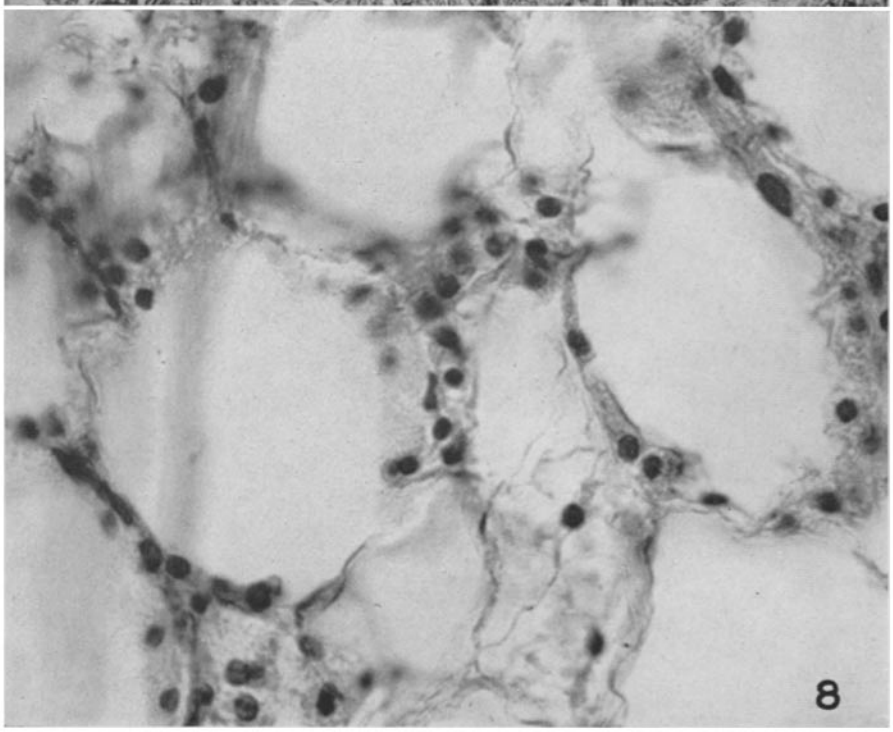


(Cooke *et al.*: Changes resembling the inflammatory in skin)

PLATE 32

FIG. 7. Human skin. Cellular increase about vessels in deep corium. × 100.
FIG. 8. Human skin. Histiocytes between fat cells in deep reticular zone. × 400.



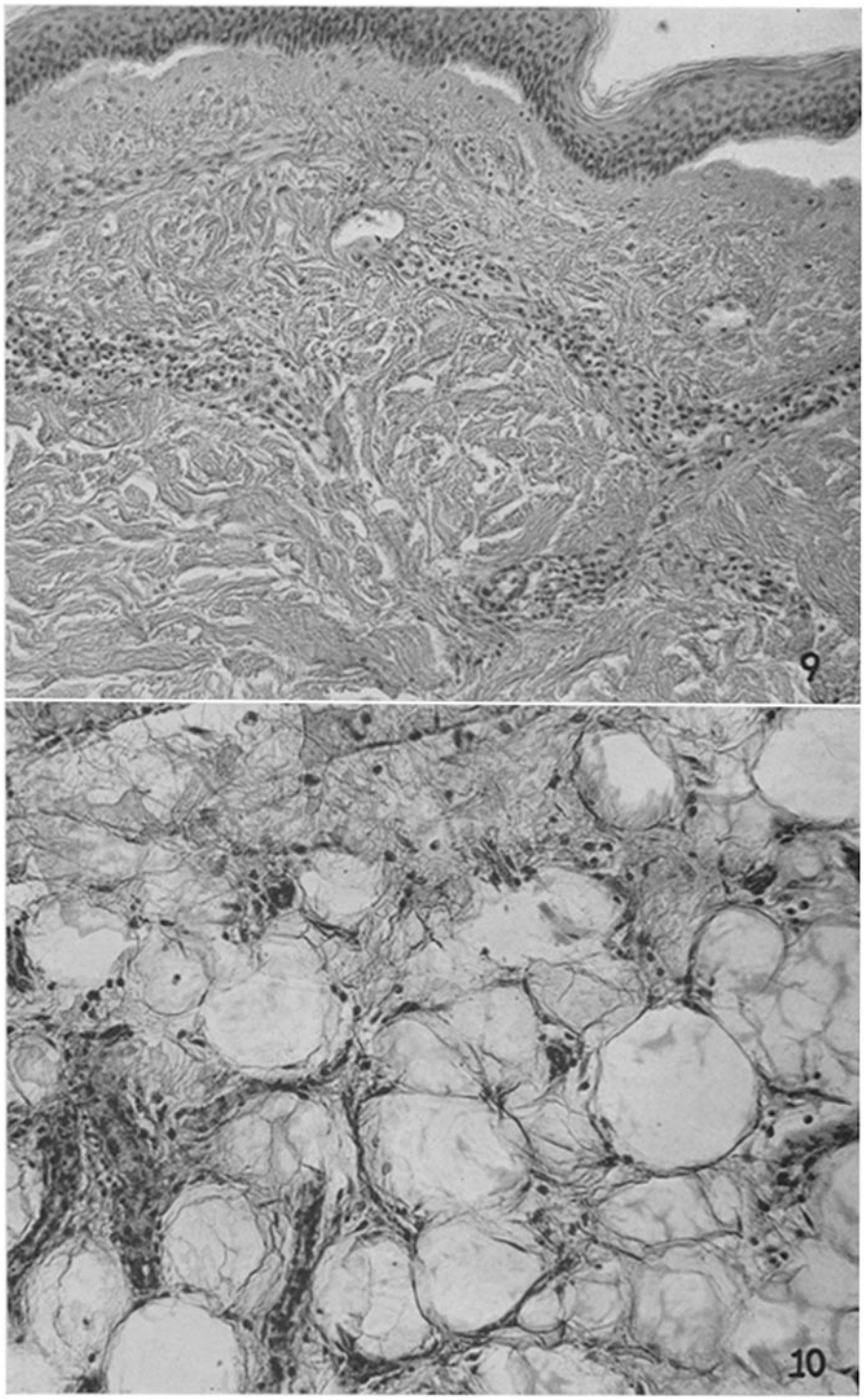


(Cooke *et al.*: Changes resembling the inflammatory in skin)

PLATE 33

FIG. 9. Human skin. From same section as Fig. 8. Cellular increase around vessels in outer papillary zone. × 100.

FIG. 10. Human skin. Cellular increase between fat cells in deep reticular zone. × 100.



(Cooke *et al.*: Changes resembling the inflammatory in skin)