gestion of 1.5 g tolbutamide(1). The difference between our findings and the results mentioned above might, however, also be due to differences in the methods used for determination of the disappearance rate.

As even a moderate inhibition of ethanol metabolism in man would be of considerable forensic interest, we feel that further investigations of this tolbutamide effect should be carried out.

Summary. With a sensitive technic it is demonstrated that tolbutamide (100 mg/kg) injected intravenously inhibits the metabolism of ethanol in intact cats, average inhibition being 34%.

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Reaction of the Subcutaneous Tissue of Rats to Injected Air.* (27123)

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The formation of localized, circumscribed pockets by injecting air or specific gases into the subcutaneous tissue of animals and man has proved to be a useful means for physiological studies of gas tissue tensions and rate of absorption of various gases from the subcutaneous tissue(1,8). These air pockets are essentially tonometers where a definite gas volume is continuously perfused by blood and the rate of exchange between gas in the pocket and the tissue-blood environment depends upon the structural and physiological characteristics of this environment. While the structural reactions of the subcutaneous tissues to air injections have been described in the guinea pig(2) and rat(3), little attention has been given to the blood supply of the air pockets which is of prime importance in such studies. It was felt therefore that air pockets in rats provide an exceptional case for study since the depot could be maintained in the same area by air refills(4) for weeks and months, thus establishing a very stable adjustment to the air as a foreign body. In fact, such gas pockets eventually form a very discrete structure with a highly developed vasculature and an organized lining. A well established pocket can actually be dissected out and removed as a complete sac, which in

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gross appearance is similar to that of the urinary bladder. The structural differentiation of such pockets as formed in rats is described.

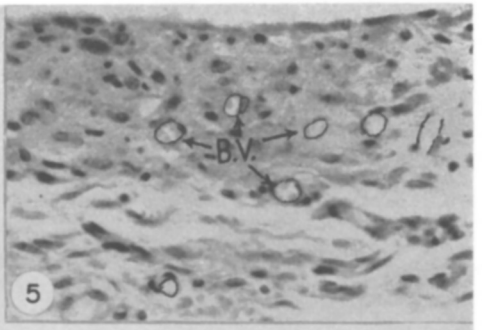
Material and methods. For measurements of gas absorption rates from subcutaneous tissue, a single, large and discrete pocket should be induced and maintained. In some strains of rats the injection of gas may produce a series of small isolated pockets or the air might distribute itself as very fine bubbles over large areas of the body. The Wistar strain was found to be satisfactory, and in our studies the Rochester strain (an inbred local strain originally derived from the Wistar strain) was used. Since the subcutaneous tissue of female rats responds better than that of males, in 43 female rats the dorsal subcutaneous tissue, subjacent to an area of skin from which the hair was removed with electric clippers, was injected *via* a hypodermic needle and syringe with 30 ml of air. Manometric measurements indicated that pressures of 10 to 20 cm H_2O were necessary to start the formation of the cavity; once started, less pressure was required to enlarge it. After formation, the pressure inside was 1 or 2 cm H_2O. No precautions were taken to filter or sterilize the air injected or to sterilize the site of injection. This strain of rats has a high degree of resistance to infections and none of the pockets became infected.

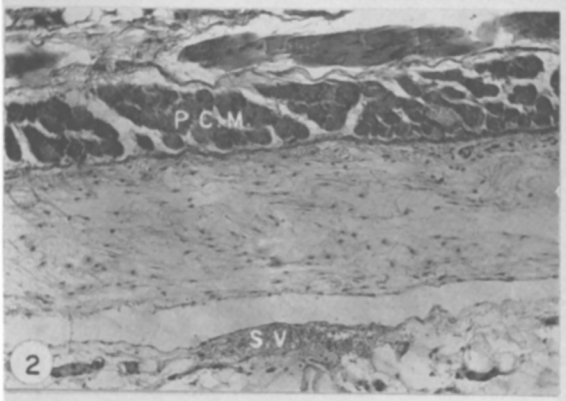
The size of the pockets was maintained with repeated air refills in order to study, at various intervals, the differentiation of the new tissues. In 15 animals, 50% India ink in saline or 8% colloidal mercury sulfide—Hille was injected into the abdominal aorta or vena cava before autopsy, to help delineate the blood vessels around the pocket. Histamine phosphate was injected into the pocket of some animals to insure better local filling of blood vessels supplying the pocket. In other animals, India ink or neutral red solutions were injected into pockets 4 to 7 days old for study of phagocytosis.

Histological sections were obtained from pockets (a): 1, 6, 18, 24, and 48 hours, and 3, 4, and 8 weeks after initial air injection; (b) from another series in which 2-week-old gas pockets were collapsed by withdrawing as much gas as possible, and tissue obtained 6 and 12 hours and 1, 2, 3, 14, and 16 days after such collapse. All tissues were fixed in formalin, and samples refixed in Bouin's or Zenker's solution for sectioning in paraffin at 7 micra. Sections were stained with Ehrlich's hematoxylin and eosin, Mallory connective tissue stain, Darrow's orcein and Ehrlich's hematoxylin, or selected sections were stained with eosin and methylene blue or Wilder's reticular stain.

Observations. Injected air formed a localized, circumscribed pocket (Fig. 1) in the dorsal subcutaneous tissue, between the panniculus carnosus muscle and the fascia over the musculature of the dorsal body wall, where it separated strands of collagenous and elastic fibers and dissected along the course of superficial vessels (Fig. 2) and nerves. During the first 2 days after injection, reaction to the injected air consisted mainly of an infiltration of polymorphonuclear leucocytes into tissue surrounding the air and accumulation of a small mount of plasma-like fluid lining the inner surface of the pocket. After 3 or 4 days, the number of polymorphonuclear leucocytes decreased and mononuclear cells invaded the pocket walls with concomitant fibroplasia, which was most active 7 to 9 days after air injection. Cells at the gas-tissue interface became flattened and spindle shaped—resembling the cells lining a synovial cavity (Fig. 3 and 5). These flattened cells apparently were modified fibroblasts but their cytoplasm was more basophilic than that of the fibroblasts in the deeper (outer) layers of the wall. Formation of the multilayered wall appeared to be due both to compression of cells by the injected air and cell proliferation—for mitoses were observed in the cells forming the wall around the air pocket even at 2 weeks after initial air injection. By the end of 2 weeks, the multilayered connective tissue wall had reached its maximum thickness (Fig. 3). Blood supply to the pocket was observed to increase in proportion to its connective tissue content (Fig. 3, 5, and 7).

When air was absorbed normally or after it was withdrawn from the pocket, the walls approximated and fused with one another,


B.V.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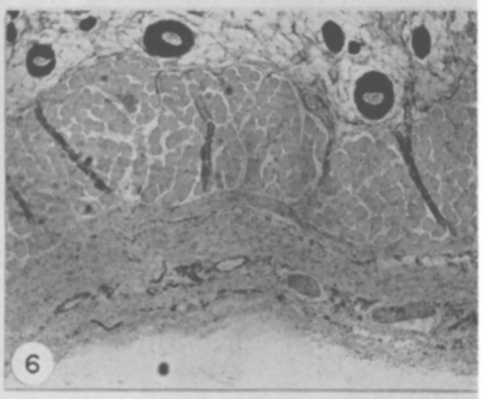
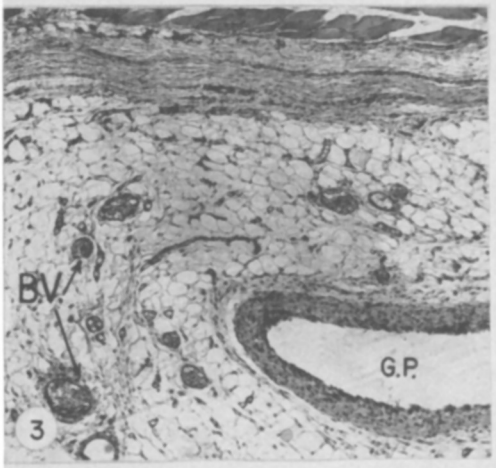
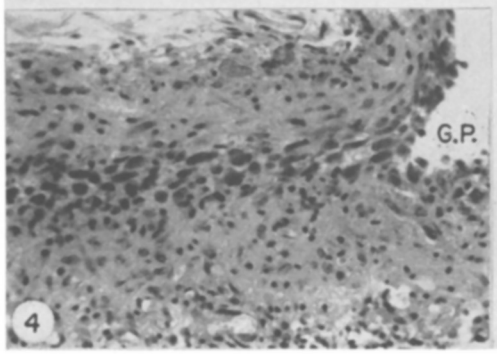



B.V.
G.P.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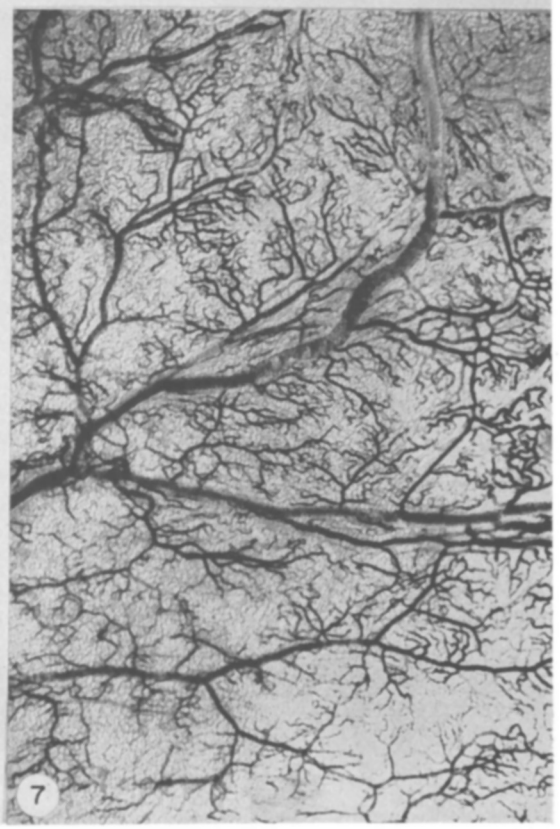

and the flat, spindle-shaped cells which lined the cavity became enlarged and rounded (Fig. 4). Within days after gas removal many of the collagenous fibers and their associated cells had been resorbed. By the end of 2 months, the only inflammatory elements remaining were the enlarged blood vessels.

Comment. Reaction of the dorsal subcutaneous tissue of the rat to injected air was similar in many respects to that described by Wright(2) for the response of the subcutaneous tissue of guinea pigs to oxygen, nitrogen, and carbon dioxide, except that in no instances were giant cells or epitheloid cells found in the area of the air injections. India ink or neutral red solutions injected into the pockets also indicate that monocytes containing these substances are formed in the walls of the pocket.

It has been observed that the partial pressures of O_2 and CO_2 in gas pockets in general (9,10) as well as in the skin pocket of rats (8,9) are similar to the O_2 and CO_2 tensions predicted for venous blood rather than arterial blood. In fact the gas pocket can be regarded as a perfect venous blood gas tonometer. This study helps to account for such findings. Arterial supply to the dorsal subcutaneous tissue is derived from: posterior auricular branch of the external carotid for the occipital region; lateral thoracic artery, from the axillary, to the lateral thoracic wall; thoracodorsal artery, from the subscapular, to the region of the scapula; and small branches from the lateral femoral circumflex of the hypogastric and iliolumbar arteries to the caudo-dorsal region. However, like subcutaneous tissue in other parts of the body, the majority of vessels were veins and anastomosing venous arcades (Fig. 7) with small branches of the arteries in the central part of the venous arcades. These superficial vessels, particularly the veins, increased in size and complexity with the duration of the air in the subcutaneous tissue. Since very few capillaries were observed in the superficial part of the pocket wall (Fig. 5), absorption of air must have occurred through several layers of cells in the pocket wall. Tissues in the wall were therefore exposed to the average gas tensions of capillary blood which approximates that of venous blood, and since the area of the veins and venules was comparatively larger than that of arteries and the arterioles (Fig. 7) in and around the walls of the pocket, the composition of the gas would most likely be nearer to venous than to arterial blood.

Subcutaneous pockets have also been used to study inflammation(5), tissue transplantation(6), and tumor growth(7) indicating that this experimental method has many uses in addition to the absorption of injected air(8).

Summary. Single discrete gas pockets were formed by injection of air into the dorsal subcutaneous tissue of rats. The presence of the gas caused a local morphological reorganization which was complete in 2 weeks. A wall, consisting mainly of modified fibroblasts and collagen fibers, developed around the gas and local vasculature increased in proportion to the wall thickness. After removal of the gas, opposing surfaces of the wall fused together and the collagen fibers with their associated

FIG. 1. Rat with circumscribed subcutaneous gas pocket × 54.

FIG. 2. Section through edge of a gas pocket, 6 hr after inj. of air. The air dissected along the course of a vein (S.V.) in the loose connective tissue below panniculus carnosis muscle (P.C.M.) × 90.

FIG. 3. Section through part of a gas pocket (G.P.) 2 wk after initial inj. Note thickness of wall and large, numerous blood vessels (B.V.) × 54.

FIG. 4. Section through gas pocket (G.P.) 12 hr after partial removal of gas from a 2-wk-old preparation. Walls have begun to fuse, and the elongated, modified fibroblasts which lined the wall have become rounded and enlarged along the line of fusion.

FIG. 5. Section through wall of gas pocket (2 wk old) showing flattened, modified fibroblasts (upper left) which form the lining, and the small blood vessels (outlined) B.V., mainly capillaries, in wall of the pocket × 216.

FIG. 6. Section through subcutaneous tissue showing remainder of wall of a 2-wk pocket from which air was removed. The fibrous tissue has been absorbed but enlarged blood vessels remain × 43.

FIG. 7. Veins inj. with colloidal mercury sulphide, in whole mount of a gas pocket (2 wk duration). Anastomosing venous arcades are numerous. The arterial supply, not readily identified in this preparation, was relatively small and entered into the tissue in the center of the venous arcades × 12.

cells were resorbed. Enlarged blood vessels remained after the other modified structures were no longer present in the pocket wall.

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Lobster Shell and Hemolymph Composition Under Normal and Acidotic Conditions.* (27124)

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The calcified portion of the shell of the American lobster, *Homarus americanus*, contains primarily calcium carbonate, with smaller amounts of sodium, potassium, phosphate and other ions present. The mammalian skeleton participates in homeostatic regulation of body-fluid ionic concentration but the effects of disturbances in acid-base balance upon the shell mineral are not clear. Collip(1) and Tilgner-Peter and Tilgner(2) suggested that the carbonate of the exoskeletons of molluscs and intermolt crustaceans could serve as a source of buffer during such disturbances. In the present study, the carbon dioxide tension of the artificial sea water environment of the lobster was increased. This permitted a study of the effects of alterations of acid-base balance on the composition of the shell and hemolymph.

Methods. Live male lobsters weighing 400-550 g, obtained from a local market, were allowed to equilibrate at least 24 hours at 8-10°C in an aerated bathing solution composed of 3.4% commercial sea salt in distilled water. The animals were not fed during the experiments.

The concentrations of several components of the hemolymph and shell of the control animals were determined. Citrate was estimated by a modification of the method of Carlsson and Hollunger(3), and the procedure of color formation developed by Taussky and Shorr(4) was employed. A step was introduced in which the heptane extract, containing pentabromoacetone obtained by oxidation of citric acid, was washed with saturated sodium carbonate solution. Hemolymph chloride was estimated as described by Schales and Schales(5). The method of Caster, MacDonald and Armstrong(6) was employed for determination of shell chloride.

Weighed portions of hemolymph and dry shell were ashed at 450-500°C and the ash was dissolved in a known volume of dilute hydrochloric acid. The sodium and potassium contents of aliquots of these ash solutions were determined, using a Coleman flame photometer. Calcium content of the ash solutions was determined as described by Clark and Collip(7). The method of Fiske and Subbarow(8) was used to estimate phosphorus. Analysis of ash solutions indicated the quantity of total phosphate, while a trichloroacetic acid filtrate was employed in determination of inorganic hemolymph phosphate.

The pH of the hemolymph in the pericardial sinus and the arthrodial joints was determined *in vivo* with a pointed quinhydrone electrode (Beckman electrode 1190-12). To estimate total hemolymph CO_2 concentration, a sample of this fluid was with-

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