water soluble ninhydrin-positive substances. The free amino acid patterns characteristic for the specific tissues were not established until after the chicks hatched.

Even though the 4 embryonic muscles were similar in composition, their characteristics changed with time, indicating that new enzyme systems had been activated. Compound CX increased in amount at the 18th day of embryo incubation; anserine and carnosine, as previously noted by Lowe(6) were first detected in the skeletal muscles of day-old chicks, and SEP appeared in the dystrophic breast muscles sometime during the first 2 weeks of post-embryonic life. Such temporal and genetic markers of muscle development may prove useful in future studies of the factors which regulate the metabolic changes associated with embryonic growth and differentiation.

Summary. The free amino acid patterns of leg and breast muscles were studied during the development of normal and dystrophic chickens from the 14-day-old embryo to the 2-week-old chick. Although these patterns changed with time little difference was found between muscles or strains until after hatching. An unidentified ninhydrin positive substance (CX) was present at high levels in the muscles of 18- to 20-day-old embryos and day-old chicks; CX rapidly declined in all but the dystrophic breast muscle by the time the chicks were 2 weeks of age. Its subsequent disappearance was accompanied by the appearance of serine ethanolamine phosphate, which occurs characteristically in dystrophic breast muscle. Taurine, also occurring at high levels in the embryo, remained high in dystrophic chick breast muscle. The results indicate that dystrophic chick muscle retains some embryonic properties and that this form of muscular dystrophy involves changes in muscle development.

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Inflammatory Effects of Pyrogenic Steroids in Animals. (30110)

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Several 5β-H steroids produce intense fever and inflammation when injected into man (1-3). The pyrogenicity of neutral steroids, such as etiocholanolone and 11-ketopregnanolone, is highly species-specific(4). This investigation was undertaken to determine whether the pyrogenicity of the acidic steroid lithocholic acid was similarly species-specific, and to examine the possible inflammatory properties of the 3 steroids in experimental animals.

Methods. Studies on inflammation were done on unrestrained animals. After shaving the abdomen, steroid solutions (20 mg/ml in propylene glycol) were injected subcutaneously on one side of the abdomen, and an equal volume of solvent vehicle on the other. Injection sites were observed for gross

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inflammation, and biopsies of steroid and control sites were taken at 48 or 72 hours from 4 animals at random in each steroid group.

Results. Rabbits. Twelve rabbits each received 5 mg/kg (total dose 12.0 to 19.5 mg) and 0.5 mg/kg (1.2 to 2.0 mg) injections of lithocholic acid with corresponding controls. In 8 there was inflammation at the steroid but not control sites; in 3 there was inflammation at the steroid sites and minimal inflammation at the control sites; and in 1 there was inflammation at the steroid sites but more intense inflammation at the larger control injection site.

Twelve rabbits received 5 mg/kg (10.0 to 18.5 mg) and 0.5 mg/kg (1.0 to 1.7 mg) injections of 11-ketopregnanolone with corresponding controls. The smaller doses produced no significant inflammation. In 3 animals there was inflammation at the 5 mg/kg site only; in 2 there was more inflammation at the 5 mg/kg site than at the corresponding control site; in 2 there was equivalent inflammation at the 5 mg/kg and control sites; in 1 there was greater inflammation at the control than at the 5 mg/kg site; in 2 there was inflammation only at the larger control site; and there was no inflammation at either site in 2 animals.

Twelve rabbits received 5 mg/kg (11.5 to 18.5 mg) and 0.5 mg/kg (1.2 to 1.9 mg) injections of etiocholanolone with controls. In 4 inflammation occurred only at one or both steroid sites; in 2 inflammation was greater at the steroid than at the control sites; in 2 there was equivalent inflammation at steroid and control sites; in 1 there was inflammation only at the control site; there was no inflammation in 3 animals.

Guinea pigs. Five guinea pigs received 5 mg/kg (1.8 mg) injections of lithocholic acid; 3 showed inflammation at the steroid injection site, and 2 had no inflammation. Five guinea pigs received 5 mg injections; 2 showed inflammation at both steroid and control sites, and 3 showed no inflammation.

Five guinea pigs received 5 mg/kg (1.8 mg) injections of 11-ketopregnanolone and 5 received 5 mg injections. No significant inflammation was observed in any animal.

Five guinea pigs received 5 mg/kg (1.8 mg) injections of etiocholanolone; 2 had inflammation at the steroid site and 3 had no inflammation. Five guinea pigs received 5 mg injections; 4 had inflammation at the steroid site and 1 had inflammation at both steroid and control sites.

Hamsters. Five hamsters received 5 mg/kg (0.6 mg) injections of lithocholic acid; no inflammation was observed. Five hamsters received 5 mg injections; 1 had inflammation at the steroid site, 1 had no inflammation, and 3 died.

Five hamsters received 5 mg/kg (0.6 mg) injections of 11-ketopregnanolone; 1 had inflammation at the steroid site, 1 had much greater inflammation at the steroid than the control site, and 3 had no inflammation. Five hamsters had 5 mg injections; 1 had inflammation at the steroid site, 3 had inflammation at both steroid and control sites and 1 had inflammation at the control site.

Five hamsters received 5 mg/kg (0.6 mg) injections of etiocholanolone; 1 had inflammation at the steroid site and 4 had no inflammation. Five hamsters received 5 mg injections; 1 had inflammation at the steroid site, 1 had no inflammation and 3 died.

Rats. Five rats received 5 mg/kg (1.5 mg) injections of lithocholic acid and 5 received 5 mg injections. Three of the former did not have inflammation; all of the remaining animals showed mild inflammation at both steroid and control sites. Histologically, however, the inflammation was much more pronounced at the steroid sites than at the control sites.

Five rats received 5 mg/kg (1.5 mg) and 5 received 5 mg injections of 11-ketopregnanolone. Similar groups received the same doses of etiocholanolone. In no instance was there either gross or microscopic inflammation.

Dogs. Six dogs received 5 mg/kg (25 mg) injections of 11-ketopregnanolone and 48 hours later biopsies were obtained. This was repeated with etiocholanolone and lithocholic acid in each animal at the same doses. Gross inflammation was not observed with injections of 11-ketopregnanolone and etiocholanolone, but acute inflammation was demonstra-

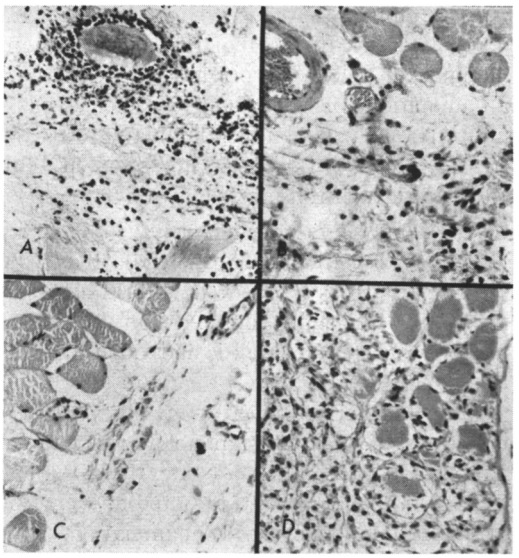

FIG. 1. A. Intense inflammation produced by 5 mg of lithocholic acid injected subcutaneously in a rat. B. Moderate inflammation at control site in the same rat. C. Absence of inflammation in a rat injected with 5 mg etiocholanolone. D. Inflammation produced by 5 mg of etiocholanolone in a guinea pig.

ble histologically at 3 of the six 11-ketopregnanolone sites and 4 of the 6 etiocholanolone sites. Intense inflammation, manifested by large (4 × 4 cm) areas of abscess formation, resulted from 4 of the 6 injections of lithocholic acid. No changes were seen at the remaining sites.

Histological changes. Histologically similar inflammation was produced by the 3 steroids at 24 and 48 hours in all species examined. Intense focal inflammation was present in some areas, with degenerating muscle fibers, thrombosed blood vessels, and sterile abscess formation. In other areas, there was a diffuse infiltration of round cells and polymorphonuclear leukocytes (Fig. 1-A and D). Control injection sites usually showed no changes, but when steroid inflammation was particularly intense, diffuse edema with some round cell infiltration was seen (Fig 1-B). Biopsies from animals with inflammation at both steroid and control sites generally showed more severe and more acute inflammation at the steroid site (Fig. 1-A and B). Inflammation produced by the smaller steroid dose was similar to but less intense than that produced by the larger steroid dose.

Temperature studies. Lithocholic acid, 5 mg/kg, was injected intramuscularly in 8 rabbits, 5 rats, 4 dogs, 4 cats and 5 monkeys. Rectal temperatures were recorded continuously with a thermister probe as described previously(4). No temperature elevations were observed in any animal.

Discussion. The results of the inflammation studies are summarized in Fig. 2. Comparison of the number of animals showing inflammation only at the steroid site with the number showing inflammation only at the control site indicates that all of the steroids tested had significant inflammatory properties. There was considerable variation, however, in species and individual susceptibility to these inflammatory effects. For example, rabbits and hamsters developed inflammation from 11-ketopregnanolone while guinea pigs and rats did not; individual dogs tended either to respond to all 3 steroids or to none at all.

Since the ability of steroids to injure cells and produce inflammation may be related more to the total amount injected than to the amount per kg of body weight, half of the animals in each group received at least 5 mg of steroid. Even with this large dose, which produces intense fever and inflammation in most humans, all 5 guinea pigs responded to

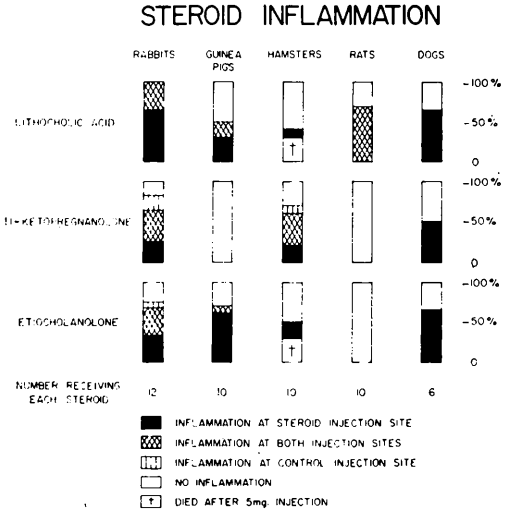

FIG. 2. Inflammation produced by 3 representative steroids.

etiocholanolone with inflammation (Fig. 1-D) but only 2 of 5 responded to lithocholic acid. Conversely, the rats responded to lithocholic acid but not to etiocholanolone (Fig. 1-A and C). The factors responsible for these variations are unknown. It is possible that the variable order of hemolytic activity exhibited by different hemolysins against erythrocytes of several species(5) may have a similar basis.

The role of the solvent vehicle in eliciting inflammation is not clear, but it seemed to be of more importance when inflammation was present elsewhere. For example, none of the injections in rats receiving neutral steroids produced inflammation, while all of the control sites in rats with lithocholic acid inflammation showed inflammatory changes (Fig. 1-C and B). Furthermore, in all species, most of the animals with inflammation at both injection sites had much more intense inflammation at the steroid site. Therefore it seems possible that in some cases the changes at the control site may actually have been secondary in some way to the intense inflammation at the adjacent steroid injection site.

Lithocholic acid, like the neutral steroid pyrogens, failed to elicit fever in animals, and therefore it does not seem possible at present to study the mechanism of steroid pyrogen activity in species other than man. It is possible, however, to study the inflammatory and cytotoxic activity of steroids in animals, and such studies may be of importance in understanding certain effects of these substances, such as their ability to produce hemolysis(6), cirrhosis of the liver(7), and other phenomena.

Summary. Three steroid pyrogens known to produce fever and inflammation in man were examined for inflammatory activity in rabbits, guinea pigs, hamsters, rats and dogs. Etiocholanolone, 11-ketopregnanolone and lithocholic acid are all capable of eliciting intense inflammatory reactions in experimental animals, although considerable variation in individual and species sensitivity was observed. Lithocholic acid, like the neutral steroids, failed to produce fever in rabbits, rats, dogs, cats and monkeys, thus confirming the highly specific nature of this activity in humans.

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Influence of Peritoneal Inflammatory Exudate on Development of a Transplantable Mouse Ascites Tumor. (30111)

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It is generally known that a more or less intense inflammatory reaction with participation of infiltrating leucocytic cells frequently accompanies growth of tumors, especially transplanted tumors. The role played by this reaction in evolution of malignant growth has been considered by many authors (1-4). The purpose of this investigation was to study the influence of inflammatory exudate produced by intraperitoneal injections

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