1) than the other two. This pattern is maintained in the dystrophic group. When the muscles in untreated normal and untreated dystrophic animals are compared, the dystrophic muscles contain less CPK, LDH and HBD, but the activity of succinate: (INT) oxidoreductase, except in the gastrocnemius, is not significantly altered. Depending on which muscle is considered, the loss in activity of CPK and LDH is between 14 and 18 per cent for LDH and between 17 and 33 per cent for CPK. The HBD–LDH ratio is also reduced by 5–7 per cent except in the tibialis anterior, the lowered value indicating a higher proportion of M-subunits.

The effect of indomethacin treatment on muscle enzyme activities in dystrophic hamsters is complex and depends upon which muscle is examined (Table 2). However, firstly, muscle enzyme levels in the treated normal group, in contrast to serum, were unaffected by the drug so that data for the normal treated group is omitted. When the dystrophic treated group is compared with the dystrophic controls, the mean tissue enzyme activities generally show a trend towards normal, but only in certain instances is the difference significant. The most striking effect is on CPK, data for which is presented separately (Fig. 1). In the gastrocnemius, for example, indomethacin treatment causes a significant rise in CPK, LDH and HBD. In all three muscles, although CPK activity in the treated dystrophic hamsters remains lower than that in the normals, the difference is no longer significant. The rise in gastrocnemius in LDH and HBD is similar, so that the ratio HBD–LDH is not significantly altered.

In the biceps femoris, there is an increase in HBD unaccompanied by any overall increase in LDH; consequently, the ratio HBD–LDH rises.

At present, there is no proof that these effects are related to the known pharmacological action of indomethacin, e.g. inhibition of prostaglandin synthetase [17] or of lysosomal enzyme release [18], so that the chief interest of the present study is the demonstration that the biochemical changes in muscle, as well as in serum, which occur in an hereditary muscle disease can be partially reversed by drug administration.

We must emphasise that, although indomethacin treatment leads to a partial restoration of intra-cellular enzyme levels to normal, we have no evidence, as yet that there is any corresponding increase in survival time of the affected animals. A pilot study now in progress may provide evidence on this point.

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Correlation between inhibition by anti-inflammatory substances, of arachidonic acid-induced hypotension and of prostaglandin biosynthesis *in vitro**

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According to Vane [1], the nonsteroidal anti-inflammatory drugs act as inhibitors of prostaglandin biosynthesis. Various studies have sought to find a parallel between the inhibition of prostaglandin biosynthesis and their antiphlogistic action, but marked differences in the inhibitory action were observed according to the organs providing prostaglandin synthetases. Therefore, no strict parallel can be drawn between synthesis inhibition and antiphlogistic effect [2, 3].

On the other hand, the hypotensive action of arachidonic acid, precursor of PGE_2 and $PGF_{2\alpha}$, is enhanced by high doses of heparin [4], whereas this hypotension is inhibited by eicosatetraynoic acid, an inhibitor of prostaglandin synthesis [5, 6]. The arachidonic acid-induced hypotension, which depends on endogenous formation of prostaglandins, could be considered as evidence of prostaglandin synthesis *in vivo*, facilitated in the presence of heparin [4]. Therefore, a study of the hypotensive activity of arachidonic acid in animals treated with nonsteroidal anti-inflammatory compounds could be used as an indicator of their inhibitory property towards prostaglandin synthetases *in vivo*. A measure of the total inhibitory activity of the anti-inflammatory drugs using intact animals could be a better approach to the actual mechanism

Table 1. Inhibitory action of several drugs on arachidonic acid-induced hypotension and on other systems described in the literature

Drug	Arachidonic acid-induced hypotension: Minimal active dose* (mg/kg)	Arachidonic acid-induced hypotension: Inhibition (%)	Vesicular synthetase† ID_{50} (M)	Carrageenin oedema‡ ID_{50} (mg/kg)	Mycobacterium arthritis§ ID_{50} (mg/kg)
L 8027	0.8 ± 0.23 (i.v.; n = 8)	100	1×10^{-5}	22	3
Indomethacin	1.84 ± 0.4 (i.v.; n = 12)	100	2.1×10^{-5}	4	0.2
L 8109	6.43 ± 0.34 (i.v.; n = 9)	100	8.3×10^{-4}	22	0.5
L 7035	70 (i.p.; n = 5) 35 (i.v.; n = 4)	0	1.7×10^{-4}	100 to 300	300
L 2197	75 (i.v.; n = 4)	0	1×10^{-4} (inactive)	100	100

* Mean ± S.D.
† From reference 7.
‡ From reference 8.
§ From reference 9.

of their antiphlogistic potency. In order to test this new *in vivo* method, we used five substances, with known effects on the biosynthesis of prostaglandins *in vitro* [7].

White Wistar rats of either sex (270–300 g) were anaesthetized with sodium pentobarbital (Nembutal, Abbott) (3 mg/100 g, i.p.). The right jugular vein was cannulated for intravenous injection and the left carotid artery to record arterial blood pressure by means of a mercury manometer. A cannula was inserted in the trachea. The rats were pretreated by intravenous injection of heparin (20 to 25 mg/kg). Dose–response hypotension curves were initially recorded for PGE_1 and PGE_2 (0.25 to 2 μg/kg). The minimal active dose of arachidonic acid (AA) was determined and repeated until a constant response was obtained. An inhibitory substance was given thereafter and the minimal dose of this substance inhibiting the vasodepressor action of AA injected in increasingly large doses up to 2.5 mg/kg was determined. Finally, the injection of a prostaglandin was repeated. Only one substance was tested by each animal.

Rabbits weighing between 2.5 and 3 kg were anaesthetized by intravenous injection of 20% urethan (1.4 g/kg). After tracheotomy, the carotid artery was cannulated in order to record blood pressure and the jugular vein was catheterized for intravenous injections. The animals were heparinized (20 mg/kg). Arachidonic acid was injected (0.15 mg/kg) before the administration of the anti-inflammatory substance, and at doses of 0.15, 0.25 and 1 mg/kg thereafter. The vasodepressor activity of PGE_2 was recorded before and after the injection of each test substance.

The following drugs were used: L 8027, 3-(2-isopropyl indolyl)-3-pyridylketone; L 8109, (5-chloro-3-methyl 2-benzo [b] thienyl) acetic acid; L 2197, 2-ethyl-3-(4-hydroxybenzoyl)-benzofuran; these substances were dissolved in polyethylene glycol–NaCl 0.9% (10:90, v/v). L 7035, 2-isopropyl 3-isonicotinoyl benzofuran, was dissolved in polyethylene glycol–NaCl 0.9% (40:60, v/v). These four drugs were supplied by Labaz, Avenue de Béjar, 1120 Brussels. Indomethacin (Indocid, Merck, Sharp & Dohme) was dissolved in a small volume of saturated sodium carbonate solution, adjusted to pH 7.4 with HCl and made to a final concentration of 1 mg/ml in NaCl 0.9%. Arachidonic acid (Sigma) was dissolved in 2 M Tris–HCl at pH 7.8 and adjusted to 0.5 mg/ml in NaCl 0.9%.

Indomethacin, L 8027 and L 8109 induced a 10 to 15 per cent increase in blood pressure for more than one hour. This increase was sometimes preceded by a transient fall, a response which was only induced by L 7035 and L 2197.

None of the substances inhibited the hypotensive action of PGE_1 and PGE_2 in the rat or rabbit. In the rabbit, L 8027, L 8109 (1.5 mg/kg) and indomethacin (4.5 mg/kg) completely inhibited the hypotensive response induced by AA (1 mg/kg). L 7035 and L 2197 (9 mg/kg) did not interfere with the hypotensive action of AA. In the rat, indomethacin, L 8027 and L 8109 suppressed the vasodepressive activity of AA. The effect was obtained in one minute and lasted for more than one hour. L 7035 and L 2197 had no inhibitory effect. Polyethylene glycol had no influence upon the hypotensive action of PGE_1, PGE_2 and AA.

Table 1 compares the doses of the substances which suppress the hypotensive activity of AA (2.5 mg/kg) in the rat with the data found in the literature on the *in vitro* inhibitory power of these five substances against vesicular synthetases [7] and also their ID_{50} against carrageenin oedema and Mycobacterium arthritis in the rat [8, 9]. There is a good parallel between the inhibition of vesicular synthetases and their action on the vasodepressive activity of AA. In both cases, the activity decreases from compounds L 8027 to L 8109. For the first three of the series the correlation coefficient is 0.98 ($P < 0.01$). L 2197 has no inhibitory action in the two tests. L 7035, as the single exception, is much weaker than indomethacin on synthesis *in vitro* but has no action *in vivo*.

This discrepancy between the *in vitro* and *in vivo* effect of L 7035, might be explained by its low water-solubility. *In vivo* the drug would not reach the synthetases involved in the biosynthesis of prostaglandins. According to Ferreira and Vargaftig [10], prostaglandin formation, which is responsible for the vasodilator action of AA in the vascular bed on the dog's leg, takes place in platelets. However, in the platelet-depleted rabbit, AA remains hypotensive [11]. Thus, PG synthesis from AA might occur in some vascular walls. The sensitivity of these synthetases towards the anti-inflammatory substances may be different from the sensitivity of the vesicular synthetases. This difference could also explain the *in vivo* inactivity of L 7035.

Table 1 shows that there is no parallel between the antiphlogistic activity of the tested anti-inflammatory drugs and their inhibitory activity on prostaglandin synthesis, either *in vivo* or *in vitro* [7, see also Ref. 12]. Moreover the ID_{50} of the inhibitors tested varies with the

selected model of inflammatory reaction. This difference could be explained, according to Vane [1-3], either by a variation in the sensitivity of the prostaglandin synthetases with respect to the anti-inflammatory drugs or by the altered role of prostaglandins in the determination of the various inflammatory responses. In the latter case, the action of the anti-inflammatory agents could be attributed to other mechanisms.

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Effect of benzo(a)pyrene and chlorpromazine on aryl hydrocarbon hydroxylase activity from rat tissues

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Aryl hydrocarbon hydroxylase (AHH), an inducible mixed-function oxidase system, is found in animal tissues, essentially those at the portals of entry to the body [1–12]. This enzyme system metabolizes polycyclic hydrocarbons and related compounds [11, 13, 14]. As polycyclic hydrocarbons are found in the environment, the metabolism of such compounds has been the object of many investigations [3, 13, 15–19]. The biotransformation process renders these compounds more water-soluble, and hence more excretable, but during this process of some polycyclic hydrocarbons, toxic intermediates may be formed [20, 21]. A polycyclic hydrocarbon, benzo(a)pyrene, has been implicated through an AHH formed intermediated, as one of the precarcinogens found in cigarette smoke and thereby a contributor to the rapidly increasing lung cancer in man [15, 16] and perhaps to carcinogenesis in other tissues. Genetic and physical factors as well as xenobiotics from drugs, dietary and environmental sources have also been shown to increase or decrease the activity of AHH [19, 22–26].

From recent studies discussed by Gillette, Mitchell and Brodie [24, 26] it appears that mixed-function oxidases of extrahepatic tissues may also be of importance in the development of serious drug toxicities through activated intermediates. Thus there is a need to examine the distribution, levels, induction response and related contribution to total body xenobiotic metabolism, of these enzyme systems.

This communication reports on the distribution and relative levels of AHH in nine rat tissues, with data on the induction response to two xenobiotics, BP and CP,* to which man may become exposed.

The materials were obtained from known commercial sources as indicated in a previous paper [23]. Dr. Harry V. Gelboin of NIH U.S.A. generously donated the 3-OH-benzo(a)pyrene.

Animals. Male rats weighing between 150–200 grams or female rats in a 250–300 gram weight range (on their 15th day of pregnancy) were obtained from the Sprague–Dawley Laboratory in Madison, Wisconsin. Housing was in overhanging steel cages (20 × 11 × 8 in.) over Sanicel as bedding and in well-ventilated rooms at 76° ± 1°F with a 12-hr light cycle. Rat chow (Ralston Purina Co. in St. Louis, Mo.) was fed *ad lib.* The experiment was started after a 5-day acclimation period.

AHH Assay. In vitro drug metabolism was assayed by a microsomal hydroxylase found in various animal species and different tissues that catalyzes the ring hydroxylation of benzo(a)pyrene yielding a mixture of hydroxylated products, the major one being 3-OH-benzo(a)pyrene.

All rats were guillotined and exsanguinated between 9:00 a.m. and 12:00 noon and tissues were used immediately after washing in cold saline. Tissues were removed and prepared in the cold as follows:

Lungs from male rats were scraped with a clean single-edged razor blade to remove connective tissues of the lobar bronchi, their major branches, and the accompanying branches of the pulmonary veins and articles. Lymph nodes from male rats were removed from the mesentery and stripped of fascia. Two rats were pooled for each assay. The submaxillary and sublingual salivary glands from two male rats were pooled for each assay. One kidney from each male rat was removed and minced for assaying. With prostate tissue, two rats were pooled for each determination. Portions of the mammary glands were removed and scraped (as done with lung) to remove connective tissue. The livers of newborn male rats from separate litters were minced into 1-cm pieces and pooled. All tissues were homogenized as previously described [23].

After removing and preparing the tissues the AHH activity was assayed using modifications of the methods of Wattenberg and Kuntzman [1, 27] as previously de-

* Abbreviations used: BP = 3,4-benzo(a)pyrene. CP = chlorpromazine.