served with other modes of inhibition [5, 13–15, 18]. Similar results have been obtained in liver slices [19]. Oligomycin-resistant ATP levels in this system are higher that those obtained with cyanide. Substrate-level phosphorylation in the Krebs cycle accouts for the ATP synthesis remaining in liver slices after inhibition by oligomycin. Since the ATP concentration in oligomycin-inhibited cells is rather similar to that obtained with other modes of inhibition like anoxia [5, 12], arsenate [5], cyanide (unpublished observations) or amytal [18], it would seem that phosphorylation in the Krebs cycle does not play an important role in maintaining oligomycin-resistant ATP levels. This fraction of ATP may not be available to energy-consuming reactions. On the other hand, it may reflect a steady state maintained by glycolysis in either oxyntic or contaminant cells, or both. In fact, these inhibition-resistant ATP levels can be lowered further by 2-deoxy-D-glucose [5].

Thus, the inhibition of acid secretion in the intact mucosa and secretagogue-stimulated respiration in oxyntic cells by oligomycin appear to be related to the inhibition of production of ATP and not to a secondary effect of this compound. Although they do not prove it, these observations support the hypothesis that ATP plays a primary role in the coupling of energy metabolism and acid secretion in the oxyntic cell.

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Effect of adjuvant polyarthritis on liver alcohol dehydrogenase in the rat

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The injection of *Mycobacterium* in oil (Freund's adjuvant) to induce polyarthritis has been shown to impair hepatic [1–5] as well as pulmonary [3] mixed function oxidase in rats. The cause of this depression of drug metabolism and its relationship to inflammatory polyarthritis have been the subjects of much speculation, such as the possible existence of a "toxohormone" [6] and the possible existence of drug metabolism depression in man [1–3]. These drug-metabolizing enzymes are located in the endoplasmic reticulum (microsomes) of the hepatocyte; it is also of importance, in exploring the effects of inflammatory diseases, to measure the activities of enzymes in other parts of the cell. The investigation presented here explores the effect of adjuvant-induced polyarthritis on alcohol dehydrogenase (ADH, EC 1.1.1.1), a soluble enzyme located in the cytosol of hepatocytes. This enzyme has been chosen because it is the principal enzyme responsible for ethanol elimination *in vivo* [7]. In addition, changes of the *in vivo* activity of this enzyme are of importance as it follows saturation kinetics during the metabolism of ethanol, until lower body levels are reached [7–9].

Female Sprague–Dawley rats (Charles-River) were received at 126–150 g of weight and were randomly assigned to cages. Six rats per cage were housed in an area of controlled light, temperature and humidity. Purina rat chow and tap water were provided *ad lib.* Adjuvant and control rats were randomly mixed in each cage in order to minimize the variations in ADH that might result from batch to batch of rats and from differing conditions during breeding and transportation.

Inflammatory polyarthritis in half of the rats was initiated on day 0 by the subcutaneous injection of 0.3 mg *Mycobacterium butyricum* (Difco Laboratories) suspended in mineral oil (5 mg/ml) into the right hind paw plantar surface, as described by Barbieri *et al.* [10]. The controls were injected with an equal volume of mineral oil (0.06 ml). Adjuvant arthritic and control rats were decapitated and exsanguinated on days 2, 7, 14 and 30. Livers were removed immediately and weighed, and sufficient ice-cold KCl solution (1.15%) was added to give a 20% homogenate (w/v). After homogenization at 4° with a Thomas glass homogenizer (type B) with a Teflon pestle, the homogenate was placed in a plastic centrifuge tube and centrifuged for 20 min at 12,500 *g* at 4°. The supernatant fraction was removed, kept at 4° and assayed immediately for ADH.

Enzyme assay. ADH was assayed as follows. The reaction mixture consisted of 1.8 ml of 0.2 M glycine buffer, pH 9.6, 0.1 ml of 2.0 M ethanol, and 1.0 ml of β-NAD (Sigma-Grade III) at a concentration of 1 mg/ml. After mixing and preincu-

Table 1. Effect of adjuvant polyarthritis on the *in vitro* activity of liver alcohol dehydrogenase

Day	N*	Alcohol dehydrogenase activity†		
		Control	Adjuvant	% Change
0	18	1.62 ± 0.06‡		
2	12	1.84 ± 0.15	1.86 ± 0.12	1.1
7	12	1.29 ± 0.16	1.55 ± 0.22	20.2§
14	18	1.59 ± 0.17	1.76 ± 0.17	10.7§
30	6	1.98 ± 0.10	2.14 ± 0.14	8.1

* Number of rats within each experimental group.
† ADH activity was calculated on the basis of μmoles/g of liver fresh weight/min.
‡ Mean ± S.E.M.
§ $P < 0.01$ (calculated by analysis of variance [12]).

bating at 37° for 1 min, the reaction was started by the addition of 20μl of the 12,500 *g* supernatant fraction. The rate of formation of NADH was measured at 340 nm in a Beckman DB-G spectrophotometer connected to a Beckman 10 inch recorder. Each sample was measured in duplicate and the average increase in the extinction was calculated on the basis of μ moles/g of liver fresh weight/min by the method of Mattenheimer [11]. A blank reaction is known to occur in the absence of ethanol [7]. Our measurements confirmed this but exhibited no difference in per cent change upon subtraction of blank values. In addition, because of the overwhelming effect of the ADH reaction, it was decided that the total value of NADH formation was the more accurate parameter.

The data on ADH activity in liver supernatant fractions of control and adjuvant rats are given in Table 1. The diseased animals had a significantly increased ADH activity of 20 per cent at 7 days and 10 per cent at 14 days after the injection of Freund's adjuvant. This time period of 7–14 days corresponds to the actively developing phase of the arthritic disease, monitored by increased hind paw size, the appearance of inflammatory nodules on the ear and tail, and depressed body weight gain and mobility observed in this and other laboratories [1–6]. Depressed body weight gain was similar in our animals to results noted previously [1–6]. In addition, as also reported previously, no significant difference in liver weight was found when comparing adjuvant to control rats.

Inhibition of the microsomal drug-metabolizing system correlates positively with the developing disseminated arthritic disease. At 7–14 days after injection of Freund's adjuvant, hepatic drug metabolism, as measured by the *in vitro* activity of aryl hydrocarbon hydroxylase, is depressed to 25 per cent of controls [3]. From these results it appears that, as the inflammatory disease progresses into the actively developed phase, the hepatic microsomal enzyme activity decreases as the hepatic ADH increases.

These data indicate that Freund's adjuvant, used in this manner, is not a non-specific hepatotoxin. This concept has been supported by the data of others. For example, Fujihara *et al.* [13] indicated that there is no gross impairment of the hepatic reticuloendothelial apparatus in polyarthritic rats. Whitehouse and Beck [14] have stated that, although microscopic lesions have been observed in the livers of diseased animals, the depression of drug metabolism is not due to liver necrosis. Carlson and Ciaccio [3] have published data indicating that, even though the base level of a drug-metabolizing enzyme system decreases drastically during this disease, both in liver and lung, the same system still responds to an inducing agent. DiPasquale *et al.* [15] have shown that serum glutamic pyruvic transaminase, alkaline phosphatase, and blood urea nitrogen were not elevated in arthritic rats; thus, commonly used "markers" failed to detect liver disease or toxicity. The well known data [1, 2] indicating increases in plasma levels and hence liver synthesis of fibrinogen and A_2 macroglobulins also support the concept that Freund's adjuvant is not a non-specific hepatotoxin.

We hypothesize that adjuvant-induced polyarthritic disease is associated with a change in the priorities of the hepatocyte. This may occur as a consequence of a "toxohormone" released during the disease process, as proposed by Fujihira and Whitehouse [5], which may inhibit the microsomal drug-metabolizing system and induce other systems such as the cytosol-localized ADH. Alternatively in this case, the elevated ADH activity may indicate a protective mechanism against toxic products accumulating as a result of a depression of some microsomal enzymes. In any event, measurements of other soluble enzymes of the hepatocyte should be performed in order to understand more fully the hepatic effects of polyarthritic disease.

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