tubation to rats with permanent thoracic duct cannulations. Corn oil administered to rats was readily emulsified, absorbed and transported to the lymph. Fifty per cent of the corn oil was absorbed in fasted rats. Maximum absorption of corn oil occurred after 8 hours, at which time 77% of the test fat was absorbed. Triricinolein and ricinoleic acid were completely absorbed after 27 hours, reaching maximum absorption within 24 hours after administration. The lymph lipids from rats fed the hydroxylated materials contained a low concentration of triglyceride and a higher concentration of mono- and diglycerides as well as free fatty acids when compared to that obtained from animals fed corn oil.

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Detection of Apotryptophan Pyrrolase Activity in Unfractionated Liver Homogenates.* (30763)

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A previous report(1) demonstrated that tryptophan pyrrolase activity of whole liver homogenates from normal mice was approximately doubled when an optimal excess of the hematin coenzyme was added to the assay mixture. Feigelson and Greengard(2) have reported that measurement of tryptophan pyrrolase activity in an unfractionated rat liver homogenate generally gave adequate estimates of the tryptophan pyrrolase content since the amount of microsomes and mitochondria in such an homogenate provided near-optimal amounts of coenzymes. In our laboratories, however, tryptophan pyrrolase activity in whole homogenates of rat liver was found to be increased when hematin was added to the assay mixture. It therefore became obvious that the ability to measure this heretofore undescribed reservoir of free apoenzyme in unfractionated liver homogenates was attributable to some variation in the assay procedure.

Methods and materials. The only deviation from the procedure described by Knox(3) for assay of tryptophan pyrrolase was in the preparation of the liver homogenate. The original procedure(3) specifies homogeniza-

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tion in a Waring blendor for 2 minutes at 2°C. In our laboratories, tissues are homogenized in a tissue grinder consisting of a piston-type Teflon pestle and a grinding vessel of unground glass(4) (Arthur H. Thomas Co., Philadelphia, Pa.). In experiments designed to compare activities of tryptophan pyrrolase in liver homogenates prepared by the aforementioned techniques, the Omni-Mixer (Ivan Sorvall, Inc., Norwalk, Conn.), rather than the Waring blendor, was utilized because of the small volume being homogenized (about 7 ml) and because its design permits submersion of the receptacle in ice-water during homogenization. Both the Omni-Mixer and the Waring blendor have rotor-knife blades of a similar design.

In all experiments, the assay mixture contained 1 ml 0.2 M sodium phosphate buffer, pH 7.0; 0.3 ml 0.03 M L-tryptophan; 1.7 ml distilled water, or 0.5 ml (10 μg) hematin and 1.2 ml water; and, 1 ml of homogenate containing whole liver homogenized in 7 volumes of cold 0.14 M KCl-0.0025 N NaOH. After incubation in a water bath-shaker (Eberbach Corp., Ann Arbor, Mich.) for 1 hour at 38°C in an atmosphere of O_2, the reaction was stopped by addition of 2 ml 15% HPO_3. This mixture was kept at 38°C for 5 minutes before it was filtered through Whatman No. 1 filter paper. To 3 ml of filtrate was added 1 ml of 1.5 N NaOH. The resulting solution was read spectrophotometrically at 360 mμ against a blank containing all components of the assay mixture except tryptophan. Hematin was prepared immediately before use by dissolving thrice crystallized bovine hemin (Sigma Chemical Co., St. Louis, Mo.) in dilute NaOH. Liver homogenates were maintained at 2°C during preparation and until added to the assay mixture.

The fraction of tryptophan pyrrolase activity detected in whole liver homogenates without added hematin is considered to be controlled by the amount of coenzyme normally present in the liver and, hence, is presumed to represent the activity of the enzyme *in vivo, i.e.,* the native holoenzyme. Estimates of free apoenzyme were obtained by subtracting the activity of the native holoenzyme from the total enzyme activity detected by adding 10 μg hematin to the assay mixture. Enzyme activity is expressed as μM kynurenine formed per hour per g liver (dry weight) during the conditions described.

Female Wistar strain rats (220-250 g) were sacrificed by a blow at the base of the skull.

Results and discussion. In preliminary experiments, aliquots of the same rat liver were homogenized at maximum speed in the Omni-Mixer and in the Teflon-glass tissue grinder until disintegration of the tissue was visibly complete. Homogenization in the Omni-Mixer was usually complete after 60 seconds; however, the resulting homogenate was never as smooth and free of tissue particles as the suspension which was completely homogenized after only 25-30 seconds in the Teflon-glass tissue grinder. Considerable foaming occurred after only 10 seconds' homogenization in the unit with rotor-knife blades, whereas the surface of the homogenate was relatively undisturbed during homogenization in the tissue grinder. Fig. 1 shows apo- and holotryptophan pyrrolase activities of whole homogenates of rat liver after visibly complete homogenization by the two techniques described. Very little apotryptophan pyrro-

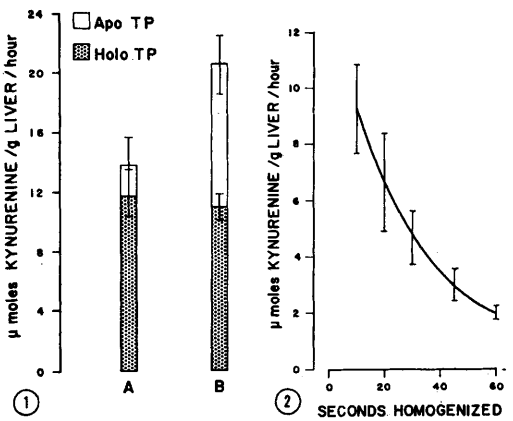

FIG. 1. Tryptophan pyrrolase activity in whole homogenates of rat liver following visibly complete homogenization in the Omni-Mixer (A) and the Teflon-glass tissue grinder (B). Rats were fasted 17 hours before sacrifice. Each bar represents the mean of 5 experiments. Vertical lines indicate S. E.

FIG. 2. Effect of homogenization in the Omni-Mixer on apotryptophan pyrrolase activity in whole homogenates of rat liver. Vertical lines indicate S. E.

TABLE I. Effect of Rotor-Knife Blade Homogenization on Tryptophan Pyrrolase Activity of Unfractionated Liver Homogenates.

Rats were fasted 17 hr before sacrifice. Each value in Table represents the mean ± S.E. of 5 experiments.

Homogenization time (sec)	Tryptophan pyrrolase activity (μM kynurenine/g liver/hr) Native holoenzyme (without hematin)	Total enzyme (+ 10 μg hematin)
10	11.2 ± 1.4	20.5 ± 2.2
20	11.2 ± 1.3	17.9 ± 2.7
30	11.6 ± 1.5	16.2 ± 2.4
45	11.3 ± 1.7	14.3 ± 2.0
60	11.7 ± 1.9	13.7 ± 2.0

lase activity was detected in homogenates prepared in the Omni-Mixer. There was no significant change in the amount of native holoenzyme activity. To determine whether the failure to detect apoenzyme activity in homogenates prepared in the Omni-Mixer was the result of enzyme inactivation or of inadequate homogenization, aliquots of a single liver were homogenized for various periods. The results of these experiments are shown in Table I. Total tryptophan pyrrolase activity decreased with increased time of homogenization, while the activity of the native holoenzyme remained constant. This indicated that it was the pool of free apoenzyme which decreased during cell disintegration (see Fig. 1). The actual decrease in activity of free apotryptophan pyrrolase during homogenization in the Omni-Mixer is presented graphically in Fig. 2. Increasing the time of homogenization in the Teflon-glass tissue grinder to 60 seconds did not reduce the total tryptophan pyrrolase activity.

The induction of tryptophan pyrrolase by its substrate is considered to be a stabilization of the enzyme with a subsequent accumulation of more total enzyme; hormonal induction by cortisone involves accelerated synthesis of the enzyme(5). It was therefore considered relevant to determine the effect of homogenization on tryptophan pyrrolase induced by cortisone and by tryptophan. Fig. 3 shows a comparison between levels of induced tryptophan pyrrolase activities in whole homogenates prepared by both homogenization techniques. The effect of ho-

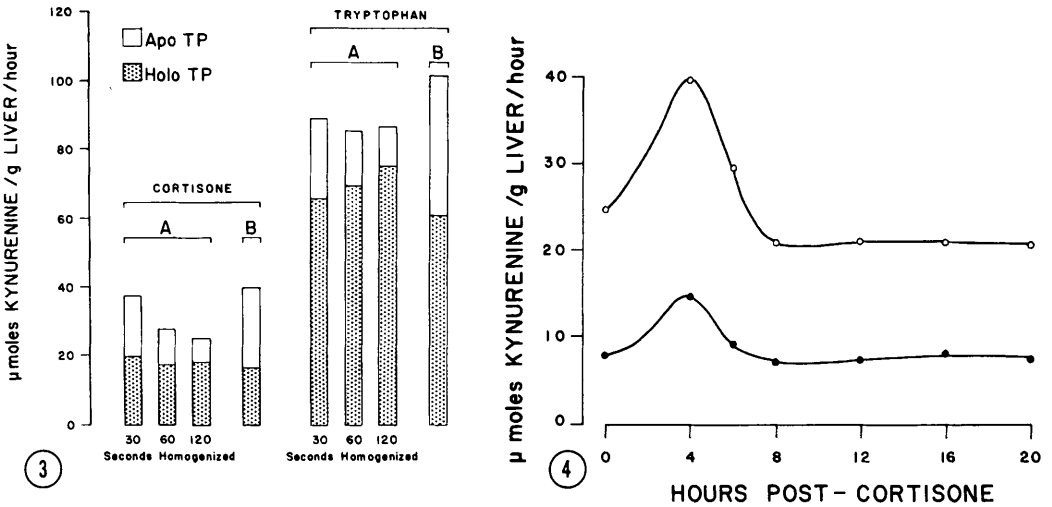

FIG. 3. Effect of homogenization on tryptophan pyrrolase activity of whole liver homogenates prepared 4 hr after injection of rats with cortisone and with tryptophan. Rats were fasted 12 hr before intraperitoneal injection of a saline suspension of 1 g L-tryptophan or 5 mg cortisone acetate (United Research Laboratories, Inc., Philadelphia, Pa.) per kg body weight. Livers were homogenized in the Omni-Mixer (A) for seconds indicated and in the Teflon-glass tissue grinder (B) until visibly complete, as described in text. Each bar represents the mean of 3 experiments.

FIG. 4. Detection of total cortisone-induced tryptophan pyrrolase activity in whole homogenates of rat liver. Rats were injected with 5 mg cortisone acetate per kg body weight at appropriate times and subsequently sacrificed together. Food was withdrawn 10 hr before sacrifice. O——O total enzyme activity (10 μg hematin added to assay mixture); ●——● native holoenzyme activity (no hematin added).

mogenization on the enzyme induced by either method of induction was comparable to that observed in homogenates of normal livers, except that the degree of inactivation of tryptophan-induced apoenzyme was not related progressively to length of homogenization time. Fig. 4 shows both native holoenzyme and total enzyme activities in unfractionated rat liver homogenates, prepared in the Teflon-glass tissue grinder, during the course of hormonal induction. No basic differences in response during cortisone induction were apparent; however, it is clearly evident that considerably more accurate estimates of actual amounts of tryptophan pyrrolase were obtained when hematin was added to the assay mixture which contained liver homogenates prepared in the Teflon-glass tissue grinder.

With the technique of homogenization described here, it has also been possible to detect tryptophan pyrrolase activity in neonatal rat liver homogenates by the third to fourth day in the absence of agents which chelate copper(6). In addition, free apoenzyme could be detected in unfractionated liver homogenates by the seventh day after birth, which is several days sooner than has been reported previously(7).

Summary. A reservoir of free apotryptophan pyrrolase has been detected in unfractionated rat liver homogenates. The detection of this enzyme fraction is dependent upon its preservation by a gentle technique of homogenizing the liver sample. The activity of free apotryptophan pyrrolase was inactivated when livers were homogenized in a unit with rotor-knife blades.

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Passive Transfer of Allergic Encephalomyelitis: Acceleration by Adrenalectomy.* (30764)

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Adrenalectomy enhances active induction of certain immunologic phenomena including experimental allergic encephalomyelitis (EAE)(1,2). Little is known about the effect of adrenalectomy on passive transfer of immunologic reactions mediated by lymphoid cells, except for a report of enhancement of graft-*vs*-host reaction(3). In the following report, acceleration of passive transfer of EAE by adrenalectomy of recipients will be demonstrated.

Methods. Lewis rats (Microbiological Associates, Inc.) with the hyperacute form of EAE(2) were donors of lymphoid cells(4). For lymph node cell transfers, donors received intraperitoneally 200 mg (wet weight) guinea pig spinal cord and 0.05 ml pertussis vaccine concentrate (10 billion organisms), homogenized and diluted with saline to 3 ml. At the first clinical signs of EAE, donors were sacrificed by exsanguination under ether anesthesia. Mediastinal lymph nodes were removed under sterile conditions, cleaned of fat, minced, and pressed and washed through a Cytosieve with saline. For spleen cell transfers, donors received an intraperitoneal in-

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