dence that in the female rat both the adrenal cortex and the adrenal medulla are essential for CPZ-induced LNPSH depletion. It is possible that any discrepancy between the evidence of Beck and Rieck(16), and the apparent necessity for a functioning adrenal medulla inferred herein may be a result of the extreme degree of stress from bilateral hindleg ligation employed by them as compared with the relatively mild stress induced by CPZ. Nagakura(17) hypothesized that severe stress can effect the release of relatively large quantities of extra-medullary catecholamines. Consequently, catecholamine release, but not necessarily a functioning adrenal medulla, may be responsible for LNPSH depletion. This is consistent with the results of preliminary experiments in our laboratories which indicate that the LNPSH lowering effect of CPZ can be prevented by pretreatment with CPZ for 5 days. Hindleg ligation-induced lowering of LNPSH was not affected with the same pretreatment.

Summary and conclusions. CPZ induced a depletion of LNPSH which differed significantly from control levels at 4 hours, but not at ½, 2, 8 and 20 hours after administration. The adrenal cortex, but not the hypophysis are integral components of the depleting mechanism. The release of epinephrine in the presence of corticosteroids may be the mediator of the CPZ-induced LNPSH depletion.

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High Specific Activity Labeling of Protein with I^{131} by the Iodine Monochloride Method.* (31148)

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Different lines of medical research and development make simple techniques desirable for attaching radioactive iodine isotopes to protein at radioactivity levels that may range for I^{131} as high as 300 mc per milligram protein. In many cases it is also essential that the methods used should result in minimal alteration of the protein either through radiation damage or by coupling an excessive amount of iodine (radioactive and non-radioactive) to the protein(1). This report describes further developments in this

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direction of the procedure of Helmkamp *et al* (2) which in turn is based on the use of iodine monochloride described by McFarlane (3). Radioactive iodine, present as iodide, is converted to $I^{131}Cl$ by chemical exchange with non-radioactive ICl. Iodination of protein can be considered to occur as a result of the following reactions:

$$ICl + NaOH \rightarrow HOI + NaCl;$$
$$HOI + \text{tyrosine residue} \rightarrow \text{iodinated tyrosine residue} + H_2O.$$

It is also shown that when there is a necessary limit to the iodine content of the final product, cognizance should be taken of the I^{127} and I^{129} that are always present in available I^{131} preparations. These former two iodine isotopes are also produced by the current methods of producing I^{131} and therefore the amounts of each should be used in any calculations involving total iodine when there is concern about possible alteration of the labeled protein by too much attached iodine. Means for minimizing the amount of non-I^{131}-iodine in I^{131} preparations are pointed out.

Materials and methods. Iodine-131. All iodinations discussed in this report deal with the use of the I^{131} isotope. Two sources of this radionuclide were used: Oak Ridge National Laboratory and Nuclear Division of Union Carbide Corp. of Tuxedo Park, N. Y. The Oak Ridge material was produced by thermal neutron induced fission of U^{235}-enriched targets; that obtained from Tuxedo Park was produced by thermal neutron activation of natural tellurium.

While one curie of I^{131} represents a mass of 8.0 μg of this nuclide, analyses of samples for total iodine, as described in detail later, always showed considerably larger relative amounts of total iodine present. The non-I^{131}-iodine present is introduced, on the basis of calculations discussed below, not by frank contamination of the product with stable carrier, but rather by the inevitable production of stable I^{127} and long-lived I^{129} (1.6×10^7 year half-life) simultaneously with that of the desired I^{131}-isotope. Both of the above-mentioned methods of production lead to these contaminating iodine isotopes, although in differing amounts, as will be discussed later.

Iodine monochloride reagent. The stock solution of ICl was 0.02 M in ICl, 2.0 M in NaCl, 0.02 M in KCl and 1.0 M in HCl. As described more fully elsewhere(2), it was prepared as follows: To a solution of 0.5550 g of KI, 0.3567 g in KIO_3 and 29.23 g of NaCl were added 21 ml of concentrated HCl (sp. gr. 1.18) and the necessary water to make the volume 250 ml. A slight amount of free iodine was removed by repeatedly shaking this solution with CCl_4, and a current of air saturated with water vapor passed through the ICl solution to volatilize suspended and dissolved CCl_4. Such a solution has a molarity within 1% of that calculated on the basis of KIO_3 used and is indefinitely stable at room temperature. Just prior to iodination of protein a subdilution of ICl is prepared in 2 M NaCl such that this solution contains in 0.2 ml the amount of ICl desired for the iodination procedure. For example, a typical procedure is to I^{131}-label 4 mg of protein of 160,000 molecular weight (2.5×10^{-8} moles) using 4 ICl molecules for each molecule of protein. These 4 equivalents of ICl in 0.2 ml are provided by a dilution of the stock ICl solution to 0.0005 M ICl (a 1 to 40 dilution).

Borate buffers. As described earlier(2) borate buffer of pH 8, designated 1X, was prepared by adjusting a distilled water solution of 0.16 M NaCl and 0.20 M H_3BO_3 with 1.6 N NaOH to pH 8 and a final NaOH concentration of approximately 0.04 M. Borate buffer with the same constituents at twice these concentrations has a pH of 7.65 and is termed 2X borate buffer.

Protein solutions. Proteins for I^{131}-labeling were prepared as solutions in 1 to 5 ml 1X borate buffer, or by dialysis against this buffer. The presence of reducing substances lowers the efficiency of I^{131} attachment to protein or prevents it altogether. As reported earlier(2), one source of such a substance is cellulose dialysis tubing. Some protein preparations can contain reducing substances not protein in nature, as complexes with proteins or impurities.

Catalase solution. Sterile preparations,† assaying 30,000 or more units per ml, were diluted to a concentration of 3,000 units per ml with distilled water. The volume of solution used for the destruction of H_2O_2, present in high activity commercial I^{131} preparations, was 0.2 ml and by Kjeldahl analysis contained 0.015 mg of protein. If use of catalase to destroy H_2O_2 is undesirable, H_2O_2 can be destroyed by addition of sulfite and excess sulfite in turn oxidized by aeration(1,2).

Resin column. Dowex 1-X4 resin (50-100 mesh) after standing in 1 N HCl for several hours was washed with 20% NaCl, then with 0.85% NaCl until the filtrate was neutral, and stored under the latter solution. A column of 2 ml of resin in a 2 ml expendable glass or plastic syringe quantitatively removed iodide ion from the iodinated protein solution.

Procedure for I^{131}-labeling. To the I^{131} solution, preferably not exceeding 3 ml in volume, contained in a standard 2 × 15 cm pyrex test tube, is added 1 ml borate buffer (2 ml if the I^{131} solution volume exceeds 4 ml) and, if the pH is not approximately 8 by wide range indicator paper, it is adjusted to this value with 1 N HCl. To this solution are then added 600 units catalase in a volume of 0.2 ml, and the mixture allowed to stand 10 minutes. Meanwhile the protein solution to be iodinated is placed in Tube C as the iodination apparatus shown in Fig. 1 is assembled. The tube containing the I^{131} (Tube A) is placed as shown in the Figure, and the I^{131} solution aspirated into and mixed with the protein solution in Tube C by briefly opening stopcock E. Tube A is now discarded. The calculated amount of ICl (0.2 ml of the requisite dilution of the stock solution with 2 M NaCl) is added to 1.8 ml 0.85% NaCl solution and the mixture similarly aspirated into Tube C. Extent and uniformity of I^{131}-labeling is dependent on rapid and vigorous mixing of the two solutions in this step. Iodination occurs rapidly(2), so 1 minute later 1 ml of some appropriate protein solution is added to the solution of iodinated protein to protect against radiation

† Sterile catalase (Code: CTS) Worthington Biochemical Corp., Freehold, N. J.

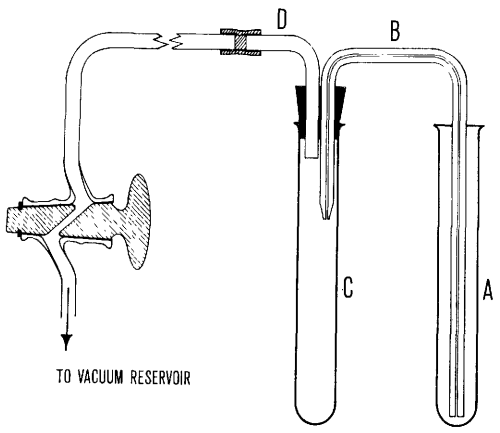

FIG. 1. Apparatus for iodination. Tubes shown at locations A and C are 2 x 15 cm Pyrex test tubes. B is a 0.1 cm internal diameter capillary tube slightly constricted at its end in C. Its effective aperture is such that application of suction will jet 5 ml of fluid from A to C in about 1 sec. Internal diameter of D is 4 mm. All operations with I^{131} are conducted behind lead shields with remote handling apparatus.

effects and the destruction of protein that may occur in dilute solutions. Typical protective proteins used in our laboratory have been a 6.25% solution of human or dog albumin, citrated plasma of human or laboratory mammal origin, normal rabbit serum, or a solution of gelatin.

If the labeled protein is not one seriously retained by the Dowex 1-4X resin, it is passed through this resin column followed by a rinse with 1.5 ml of the protective protein solution used earlier in the procedure. If the iodinated protein is one retained by the resin, *e.g.*, insulin and glucagon, unbound I^{131} is removed by dialysis, usually after addition of more protective protein.

Determination of iodination efficiencies. By the use of a NaI scintillation crystal surrounded by 4 inches of lead except for one 2 × 2-inch non-shielded area leading into the crystal, radioactive samples were counted after they were placed in a movable holder that was attached to a platform extending out from the non-shielded area of the crystal. Various thicknesses of lead plate could be inserted in front of the crystal opening. This made it possible to measure activities ranging from several microcuries to 500 millicuries. For most of the high level studies reported

TABLE I. Total Iodine Content of NaI^{131} Preparations from Oak Ridge National Laboratory. Effect of this iodine on I^{131}-iodination efficiency during iodination of 4 mg γ-globulin with 0.1 μmole of ICl at pH 7.5-8.

I^{131} preparation No.	I^{131} used in iodination (mc)	Iodine content of I^{131} preparation (μg/100 mc)	I^{131}-iodination efficiency found (%)	Predicted I^{131}-iodination efficiency (%)
1	75	2.4	55.8	56.9
2	84	34.0	19.1	20.1
3	113	6.8	38.3	40.5
4	113	6.2	39.5	41.9
5	200	2.4	44.6	47.2
6	240	2.4	45.9	44.5
7	192	3.0	44.6	44.8
8	223	3.8	39.6	38.8
9	96	23.2	23.4	23.7
10	280	2.6	43.7	41.4
11	28	3.7	59.5	60.1
12	243	14.5	15.3	17.2

here the I^{131} samples were placed about 60 cm away from the crystal and had an attenuation insert of 1¾-inch lead. Efficiency of I^{131} iodination was determined by calculating the per cent of the I^{131} initially present in the iodination tube (Tube A, above) that was recovered after passage through the resin.

Results. Much of the experience in this laboratory has been with I^{131}-labeling of γ-globulin, from normal or immunized animals, or as a purified antibody obtained by immunological techniques. When 4 mg amounts of such a protein were iodinated with an amount of ICl corresponding to 4 iodine atoms for each protein molecule of assumed 160,000 molecular weight, and the I^{131} used did not exceed 4-5 mc, efficiencies of coupling I^{131} to protein of more than 60% were almost invariably obtained, even when the use of catalase or other techniques for destroying H_2O_2 were omitted.

With amounts of I^{131} larger than 10 mc, I^{131} iodination efficiencies in runs in which no provision was made for H_2O_2 destruction often gave low values. Experiments showed, however, that by the procedure outlined in this paper efficiencies of 60% or better were again attained with amounts of I^{131} ranging from 30 to 50 mc.

With still larger amounts of I^{131} (90 to 250 mc) efficiencies achieved under conditions designed to eliminate H_2O_2, although usually substantial, showed a wide variability. A series of analyses for total iodine of highly radioactive Oak Ridge I^{131} shipments showed that efficiency of I^{131} incorporation in 4 mg portions of γ-globulin showed a strong inverse correlation with the total iodine content of the I^{131} preparation used for iodination. In the iodination procedure described here the production of $I^{131}Cl$ for labeling occurs as an exchange reaction of I^{131}-iodide ions with non-radioactive ICl. Only if the total iodide is small compared with the iodine content of the ICl will nearly all of the I^{131} be incorporated in the ICl and become available for iodination. Under the conditions studied, F, the fraction of I^{131} present as ICl when equilibrium is reached in the exchange reaction, is

$$F = \frac{12.7\ \mu g}{12.7\ \mu g + (\mu g \text{ of iodine in } I^{131} \text{ solution})}$$

where 12.7 μg is the weight of iodine in the 0.1 μmole of ICl used in these studies. Twelve total iodine determinations were made of high activity I^{131} samples from Oak Ridge that were used for I^{131} tagging of 4 mg portions of antibody to human fibrin isolated from immunized rabbits, *i.e.*, rabbit γ-globulin. Data from these studies are presented in Table I. The predicted I^{131}-iodination efficiency given in this Table is the product of F, calculated from the measured total chemical iodine content of each preparation used for iodination, multiplied by 65%, the usual I^{131}-iodination efficiency achieved with small I^{131} samples, but with the same remote handling procedures used with highly radioactive samples, where some I^{131} losses occur during transfers.

It is clear that a factor determining the amount of I^{131} coupled to protein is the total chemical iodine content of the sample used for I^{131} coupling. Total iodine contents of preparations 1 through 5 were determined by the photometric method of Sandell and Kolthoff(4) modified for analysis of highly radioactive samples. Preparations 6-12 were analyzed by a modification of Winkler's method developed for determination of iodine in I^{131} samples of high specific activity(5). A similar correlation was found between total iodine content and I^{131}-iodination efficiency with I^{131} prepared by neutron activation of tellurium.

With the recognition that high specific activity labeling of γ-globulin with minimal total iodine incorporation in protein is dependent upon the use of fresh, fission produced I^{131}, it has been possible routinely to obtain high specific activity labeled preparations without preliminary iodine analysis. In 18 such iodinations of 4 mg portions of γ-globulin carried out in the past 2 years, using I^{131} in amounts ranging from 200 to 250 mc, the per cent of I^{131} attached to protein ranged from 40 to 50%.

Discussion. Destruction of H_2O_2 by catalase. A major change from previously published methods utilizing ICl(2,3) is the addition of the I^{131} as iodide to the protein solution to be iodinated followed by addition of ICl, rather than adding to the protein solution a previously prepared I^{131}-ICl mixture. This new procedure is made feasible by discovery that the exchange reaction between iodide ion and ICl occurs at such a rapid rate that it is essentially complete before appreciable iodination of protein has occurred. This new order of adding reagents has 3 advantages. (a) Negligible ICl is lost by disproportionation to I_2 and IO_3. (b) It makes possible the use of catalase to destroy H_2O_2, since the catalase is mixed with the protein to be iodinated before iodination occurs, and under most circumstances, since the relative amount of catalase is small, the amount of labeled catalase will be negligible. (c) Addition of the ICl to the protein-NaI^{131} solution reduces to a large extent the detrimental effect of H_2O_2 on the I^{131}-iodination efficiency when no measures are taken to destroy the H_2O_2 prior to iodination. Iodination and ICl reduction by H_2O_2 become competitive. Thus, in a run at a tracer (fraction of a microcurie) level of I^{131} activity in which ICl was added to the NaI^{131} solution, and the mixture then introduced into the protein solution, the iodination efficiency was 65%. Addition of a small amount of H_2O_2 to the iodide solution reduced the efficiency of a similar iodination to 9.6%. When the ICl was jetted into a protein, I^{131}-iodide, H_2O_2 mixture containing the same amount of H_2O_2, an efficiency of 50% was achieved.

Production of I^{131} with minimum total iodine content. Fission iodine. The fission product yield from thermal neutron induced fission of U^{235} rises sharply through the regions A-127 to 133 and reaches a broad maximum about A=139. The yields for the masses of interest here are as follows: A=127, 0.13%; A=129, 0.8%; A=131, 2.93%. The iodine isotopes of these mass numbers are stable I^{127}, long-lived I^{129} (1.6×10^7 year half-life), and I^{131} (8.05 day half-life); these are formed primarily by β-decay of lower atomic number isobaric fission products. The growth equations for these iodine isotopes may be established from the complete decay schemes presented in Table III of Katcoff's recent report(6).

All other iodine isotopes produced by fission have sufficiently short half-lives that their activities will decline to negligible values after a few days cooling-off period following activation. On this basis, if separation of iodine is performed immediately after activation and is followed by a cooling-off period of 8.05 days, the calculated relative amount of iodine isotopes will be as shown in Table II.

Twenty-one recent shipments of I^{131} made to our laboratory of fission product I^{131} soon after processing contained by our measurements one day after arrival an average of 2.45 μg total iodine per 100 mc I^{131}. Values ranged from 1.6 to 4 μg. Although activation and chemical processing schedules vary from time to time, calculations indicate that most of the non-I^{131}-iodide content of these shipments is produced by fission rather than de-

TABLE II. Calculated Relative Amounts of Fission Product Iodine Isotopes Present 8.05 and 32.2 Days After Bombardment of U^{235} in Iodine Separated Immediately After Activation.

Activation time (days)	Time after activation (days)	% of total iodine I^{127}	% of total iodine I^{129}	% of total iodine I^{131}	μg total I per 100 mc I^{131}
1	8.05	0.1	22	78	1.04
10	8.05	3	34	63	1.3
20	8.05	5	42	53	1.5
40	8.05	8	55	37	2.2
1	32.20	0.4	68	31	2.6
10	32.20	7	76	17	4.7
20	32.20	10	78	12	6.6
40	32.20	12	81	7	11.6

TABLE III. Calculated Relative Amounts of Neutron Induced Iodine Isotopes Present 2 and 8.05 Days After Activation of Natural Tellurium in Iodine Separated Immediately After Activation.

Activation time (days)	Time after activation (days)	% of total iodine I^{127}	% of total iodine I^{129}	% of total iodine I^{131}	μg total I per 100 mc I^{131}
1	2	44	23	33	2.5
10	2	61	20	19	4.2
20	2	66	20	14	5.9
40	2	70	22	8	9.6
1	8.05	51	26	23	3.6
10	8.05	67	21	12	6.7
20	8.05	69	22	9	9.3
40	8.05	72	23	5	15.5

rived from contaminating stable I^{127} initially present as an impurity in the uranium or reagents used in chemical processing.

It should be noted that it is customary for some suppliers to prepare initially a large batch of I^{131} (with the inevitable I^{127} and I^{129}) and to dispense from this one lot for a period of several I^{131} half-lives. Since these old I^{131} preparations will contain much higher ratios of total iodine to I^{131}, it is important to specify recently produced I^{131} for high level labeling procedures.

I^{131} produced from tellurium. In the production of I^{131} by thermal neutron activation of natural tellurium, the stable tellurium isotopes of interest are Te^{126}, Te^{128}, and Te^{130}. The iodine isotopes produced are respectively stable I^{127}, I^{129} (1.6×10^7 year half-life), and I^{131}. From published activation cross sections(7) and decay schemes(6) one may calculate the relative amounts of different iodine isotopes present at various times after neutron activation of natural tellurium. Results of such a calculation are presented in Table III. By comparison with Table II it will be noted that reasonable irradiation and processing schedules will give I^{131} preparations with larger total iodine content than would be expected in recently prepared fission product I^{131}. This is in accord with analytical results in our laboratory.

A technique not yet explored as to feasibility and cost would be the use of tellurium enriched in Te^{130} for I^{131} production by slow neutron bombardment. A product containing 96% Te^{130} is available from Oak Ridge National Laboratory. Calculations indicate that neutron activations would yield a product substantially lower in relative I^{127} and I^{129} content than the best available fission product I^{131}.

Comparison with other I^{131}-labeling techniques. In I^{131}-labeling procedures in which iodide is oxidized to HOI or I_2 (*e.g.*, the chloramine T method) the presence of iodide other than I^{131} does not decrease the percentage of I^{131} coupled to protein, as in the ICl procedure(8). However, in labeling at high I^{131} levels the total iodine will be unknown unless a preliminary iodine determination is carried out on the I^{131} preparation to be used, or its iodine content deduced from previous

determinations made on similar preparations from the same source.

The fact that currently available I^{131} shipments frequently contain non-radioactive iodine in amounts that may exert an important effect on preparations of highly radioactive proteins seems not to be well recognized by many investigators in this field. For example, Day and co-workers(9) do not take this into account in calculating the possible limits of I^{131}-labeling of antibody protein. Also Yalow and Berson(10) seem not to recognize the possible importance of this additional iodine in the preparation of highly radioactive insulin. The detrimental effect of more than one atom of iodine coupled to an insulin molecule on the biological activity of labeled insulin has already been reported(1). Johnson *et al* (11) have shown that the coupling of more than 2-3 atoms of iodine to antibody molecules exerts significant effects upon their immunological activity. Alteration in the physiological behavior of fibrinogen tagged with more than 1-2 atoms total iodine per molecule has been demonstrated(12,13). Presumably similar effects occur in the biological activity and immunological reactivity of enzymes, hormones, and other proteins. The iodine monochloride method described here has a built-in safety device as far as chemical damage from too extensive iodination is concerned, since the iodine available for iodination cannot exceed the iodine content of the added ICl. Also a possible detrimental effect of oxidizing agents such as chloramine T on the protein to be labeled is avoided. Cohen and co-workers have shown a significant effect of some iodination procedures, independent of the total iodination achieved, on the physiological behavior of other labeled plasma proteins(14).

Radiation effects. Proteins are particularly susceptible to radiation damage in dilute solution since the ratio of free radicals and peroxides produced from irradiation of water to protein molecules is high. At a level of 10 mc/ml the self-irradiation from I^{131} is at a level of about 4,000 rad/hr. It was found earlier that a few thousand rad to antibody in dilute solution produced a significant reduction in antibody activity(2). Studies on high I^{131} level iodination of insulin emphasize the large proportion of insulin that can be altered by irradiation(10). It is important, therefore, once the iodination reaction is complete to use immediately such protective measures as addition of non-specific inert protein, dilution, and freezing, that are compatible with the intended use of iodinated protein to minimize or make negligible such radiation damage. Effectiveness of these measures with insulin has been described(1).

Summary. A modified iodine monochloride method suitable for preparing I^{131}-labeled proteins of a high degree of radioactivity is described, and results are given. Hydrogen peroxide present in high level I^{131} preparations is destroyed with catalase. Then ICl is added to a mixture of the I^{131} as iodide and the protein to be iodinated. Total iodine content of I^{131} preparations sets a limit on the specific activity of I^{131}-labeled proteins that can be achieved with a low degree of iodination. In the two commonly used methods for producing I^{131} (fission of U^{235} and thermal neutron irradiation of natural tellurium) stable I^{127} and long-lived I^{129} are also formed. Analysis showed the total iodine content of fission product I^{131}, as received from Oak Ridge National Laboratory soon after processing, to average 2.4 μg per 100 mc, 3 times the amount present as I^{131} (0.8 μg/100 mc). For I^{131} produced from tellurium it was substantially greater. Since the ratio of total iodine to I^{131} increases with time after processing, freshly produced I^{131} is necessary to make very high level labeled preparations. Precautions to prevent protein damage as a result of high level labeling procedures are described.

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Induction of Tryptophan Pyrrolase Activity in Starving Rabbits of Different Ages.* (31149)

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Previous studies(1-3) have shown that liver tryptophan pyrrolase activity (LTP) is increased in female rats and rabbits subjected to total starvation. In adult female rats(2) a bimodal response occurs with peak activities at 4 hours and between 2-7 days after the animals were placed on a starvation regimen. In young adult female rabbits(1) the bimodal response occurred within 18-36 hours and after 7-11 days from the beginning of starvation. In contrast to these findings, LTP activity in adult male rats remains relatively constant during 8-13 days of starvation(2,4). More recent experiments have demonstrated that induction of LTP activity by starvation or by injection of L-tryptophan in male and female rabbits is a function of age of the animals.

Experimental methods. Young 30-day-old male and female New Zealand rabbits weighing about 600 g were obtained commercially. The animals were maintained in the laboratory for all experiments up to 90 days of age. Older animals ranging in age between 4-5 months (80%) and 10 months (20%) were obtained commercially and distributed throughout the experiments. The data for 4-10-month-old animals were pooled because no significant differences were apparent between these ages. All animals were maintained in individual cages on commercial rabbit chow at 74°F.

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Rabbits were starved by complete removal of food but water was available *ad libitum*. For substrate induction experiments, the animals were sacrificed 6 hours after intraperitoneal injection with 0.5 g L-tryptophan per kg body weight suspended in distilled water. Control animals were injected with distilled water. In all animals, the injection volume was limited to 10 ml per kg body weight.

After sacrifice by cervical fracture, the livers were removed, chilled in ice and LTP activity determined as previously described in detail(1) by measuring the rate of kynurenine formation during a 4-hour incubation period with the diazotization procedure of Knox and Mehler(5). Exogenous hematin was not added to any of the assays(6). Liver protein was determined in the homogenates by micro-Kjeldahl procedures using 6.25 as the conversion factor. Liver protein determinations were omitted for animals injected with L-tryptophan. As previously shown(1-2), expression of enzyme activity based on wet liver weight or protein yields essentially comparable results.

The data for "2 kg" female rabbits are presented for comparative purposes and are taken from Rosenthal, Barack and Haessler (1). These animals are estimated to be approximately 60-75 days of age but the exact birth dates are unknown. Due to an error in arithmetic, the values presented in(1) are too high by a factor of 0.65 and the data shown in the present report have been cor-