tracted from the value of each test titration to give the assay value due to lipase activity.

Results. Dissection of rats 48 hours after injection with triolein revealed lungs with hemorrhages and increased weight over the lung weight of the paired control. No hemorrhages were noted in the lungs of any of the control animals. The hearts of triolein injected rats did not differ from those of the paired controls either in appearance or in weight.

Table I gives the lipase assay values in ml of 0.05 *N* NaOH per ml extract or serum. One ml extract represents 1/16 of the activity of the heart or lung. Lipase tests on the sera indicated slightly higher activity in the injected rats; however, the difference was not statistically significant. Lipase tests on the heart showed no significant difference between the triolein injected and the paired control rats. Lung lipase values of the triolein injected rats, however, were significantly greater than those of the paired controls, using the Wilcoxon matched-pairs, signed-ranks test, $\alpha = 0.005$(5). It will be noted that one control serum (Pair #15) and one control lung (Pair #5) are unusually high. The reason for this is unknown. Both of these saline-injected rats had lungs which were clear of hemorrhage and within the control weight range; therefore, they were included in the series.

Summary. The gross and histological changes in the lungs of rats injected with triolein are similar to those seen in the lungs of patients dying with fat embolism. An elevation of the serum lipase activity in patients with fractures is diagnostic of pulmonary fat embolism(6). The result of this experiment indicates that there is an increase in the amount of lipase activity in the lung in experimental fat embolsim in rats, and emphasizes the role of enzymatic breakdown in the removal of embolic fat from the lung. It also suggests that the source of the excess lipase in the serum may be the lung.

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Trace Proteins in Biological Fluids. IV. Physicochemical Properties and Sites of Formation of γ-Trace and β-Trace Proteins.* (31897)

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Two proteins of beta and gamma mobility were initially described as occurring in higher concentration in human cerebrospinal fluid (CSF) than in serum(1). Subsequently, these globulins were further characterized by the effect of enzymes and storage upon their electrophoretic mobilities(2), and behavior on column chromatography(3). The concentrations of β-trace and γ-trace were determined in the CSF of patients with and without neurological disease. It was concluded from these studies that the γ-trace protein represented approximately 2% and the β-trace 10% of

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the total CSF proteins. The β-trace protein can thus be considered to represent a major CSF protein(4).

The present experiments were carried out to determine some additional physicochemical properties of β-trace and γ-trace after purification by column chromatography. In addition, the sites of formation of these proteins were studied in an attempt to ascertain whether these proteins were products of the central nervous system.

Materials and methods. γ-Trace and β-trace proteins were isolated from the CSF of patients without neurological disease by column chromatography as described previously(3). In addition, some preparations of the trace proteins contaminated with gamma globulin, after initial separation with DEAE-cellulose chromatography of whole CSF, were purified on Sephadex G-75 according to the technique described by Link(5). Preparations used for determination of extinction coefficients contained a single protein as shown by double diffusion in agar(6). Spectrophotometric readings were done on a Zeiss spectrophotometer PMQ-2. Ultra-violet and Biuret extinction coefficients for each protein were determined(7). The nitrogen content was determined by the Markham microkjeldahl method(8).

Ultracentrifugation studies on both proteins were carried out with a Spinco analytical ultracentrifuge. The protein concentration was initially 5 mg/ml in 0.15 M NaCl.

Sites of formation of trace proteins were studied by determination of C^{14}-amino acid incorporation into these proteins by tissues cultured *in vitro* as described previously(9). 50-100 mg of tissue were cultured for 24-48 hours in a medium containing C^{14}-labeled lysine and isoleucine (1 μc of each amino acid per ml). The tissue culture fluids were concentrated by lyophilization and analyzed by immunoelectrophoresis and autoradiography(9).

Saline extracts were made from Rhesus monkey tissues in a Potter-Elvehjem homogenizer at 4°C. All extracts were lyophilized and redissolved to approximately 10% of the original volume of tissue.

Results. Physical chemical properties of purified trace proteins. Fig. 1a shows the sedimentation pattern of β-trace protein in saline after 94 minutes at 59,780 rpm. Two sedimentation peaks are observed. The sedimentation coefficients of these two components are 2.1 and 0.5 Svedberg units. Since immunization with this purified protein does not lead to detectable precipitating antibodies other than anti-β-trace, it is possible that the larger molecular weight component is a polymerized form of the smaller component.

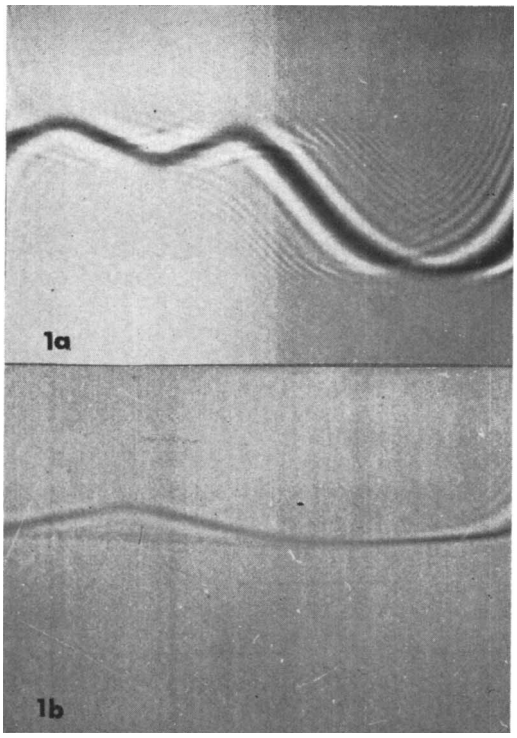

Fig. 1. Schlieren pattern during sedimentation velocity of β- and γ-trace proteins in 0.15 M NaCl; initially 5 mg/ml; 94 min after reaching 59,780 rpm, migration from left to right; temperature 21.6°C; phase plate angle 70°. Sedimentation rates for β-trace components are $S_{20,w}=0.5$ and $S_{20,w}=2.1$ (a) and for γ-trace $S_{20,w}=1.6$ (b), assuming $\bar{V} = 0.74$.

Fig. 1b demonstrates the sedimentation behavior of γ-trace protein, analyzed in the ultracentrifuge under identical conditions. Its sedimentation coefficient is 1.6 Svedberg units. No other sedimentation peaks were observed in this preparation.

Table I summarizes the results and shows extinction coefficients for ultra-violet absorption of solutions of these proteins in 0.25

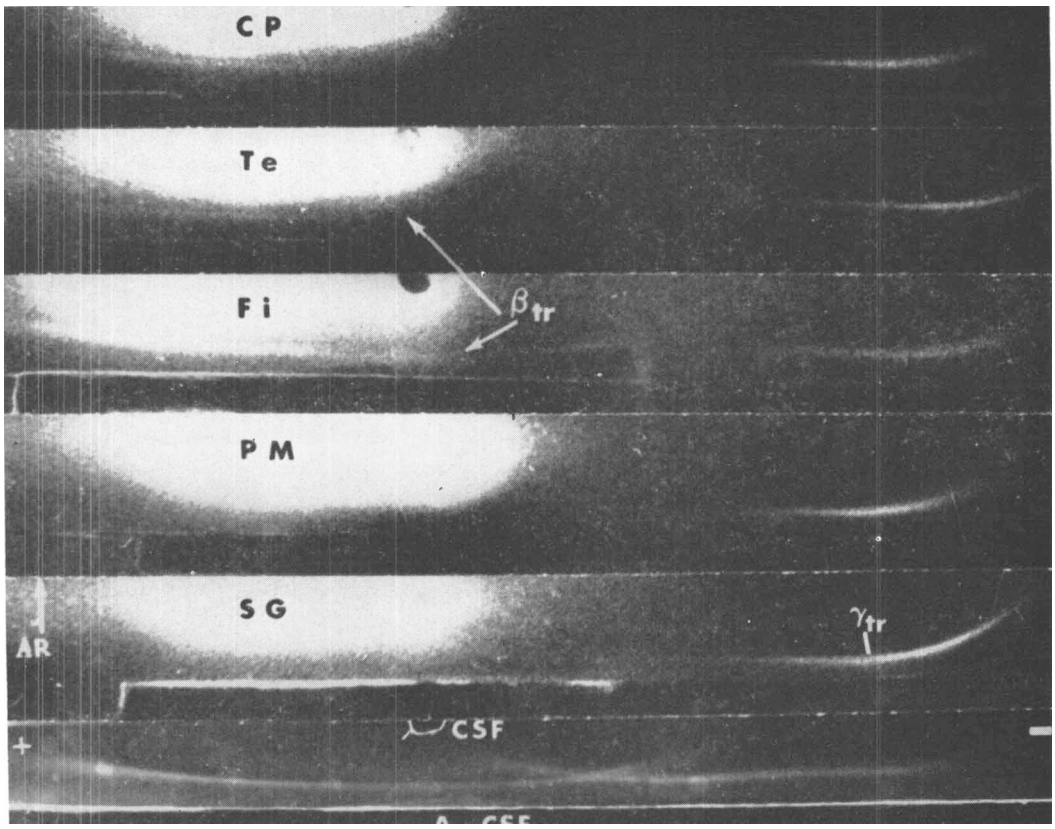

Fig. 2. Autoradiographs (AR) of immunoelectrophoretic patterns prepared with culture fluids of various monkey tissues. The patterns (bottom) were prepared with human serum as the carrier and developed with an absorbed antiserum to cerebrospinal fluid showing only β- and γ-trace. Note labeling of the slow portion of γ-trace in culture fluids of submaxillary gland (SG), peritoneal macrophages (PM), fimbriae of Fallopian tube (Fi), testis (Te), and choroid plexus (CP). β-Trace is labeled only in the latter three.

M acetic acid at 277 mμ. Extinction coefficients for protein determinations by the Biuret reaction are also given. β-Trace protein in solution had a slightly yellow color.

Sites of production of the trace proteins. Monkey tissues were used in these studies. Monkey CSF gives a precipitation line with anti-human γ-trace on double diffusion in agar which shows immunological identity with the line formed with human CSF. Strong precipitation lines, typical of both trace proteins are shown upon immunoelectrophoresis of concentrated monkey CSF developed with antisera against human trace proteins. The incorporation of C^{14}-amino acid into trace proteins was therefore examined with the aid of immunoelectrophoretic patterns, using 100 times concentrated human CSF as the carrier protein and anti-human CSF, as well as specific anti-β or γ-trace as the antisera.

TABLE I. Physicochemical Characteristics of γ-Trace and β-Trace.

		β_{tr}	γ_{tr}
Sedimentation coefficient	($S_{20,w}$)	2.1; 0.5	1.6
E^{555}Biuret	$\left(\frac{OD}{\mu gN/2.5\ ml}\right)$	.00094	.00069
E^{277}	$\left(\frac{OD}{\mu gN/ml}\right)$	.0152	.0090

Various monkey tissues were examined for production of γ- and β-trace (Fig. 2). Results are summarized in Tables II and III. It can be seen that many tissues label the γ-trace protein. The previous results obtained with

TABLE II. *In vitro* Synthesis of γ-Trace by Various Rhesus Monkey Tissues.

Tissue	Total No. cultures	Degree of labeling —	w+	+	++
Subm gland	9			1	8
Parotid gland	4			3	1
Mamm gland	4			3	1
Thyroid	8	2	2	3	1
Testis	1				1
Fimbriae ov	4		1	3	
Ovary	8	5	1	2	
Liver	11	10	1		
Kidney	9	5	1	3	
Small intest	6	5		1	
Spleen	10	2	1	7	
Lymph node	10	2	3	5	
Bone marrow	7	2	2	3	
Macrophages	4		2	2	
Periph leuk	3	3			
Brain	12	12			
Choroid plexus	5	1	2	2	

Degree of labeling was graded from w+ to ++ according to intensity of autoradiographic image.

human tissues were confirmed in that lymphoid tissue and bone marrow, but not peripheral blood leukocytes label γ-trace(10). Even stronger labeling was obtained in cultures of various epithelial tissues, among which the salivary glands, especially the submaxillary, showed the most consistent and strongest labeling (Fig. 2). Liver of adult animals never showed detectable incorporation of C^{14}-amino acid into γ-trace. One of the two culture fluids from fetal livers had weak γ-trace labeling. Brain cultures were also negative but the choroid plexus did produce some degree of γ-trace labeling in 4 out of 5 animals. Several cultures of peritoneal macrophages also showed γ-trace labeling. The cathodal portion of γ-trace usually showed stronger labeling than the anodal part (Fig. 2). The electrophoretically slow component is the only one present in CSF before storage at −20°C(2).

Saline extracts of a few monkey tissues including the liver, brain, spleen and submaxillary gland were examined for the presence of γ-trace. The only tissue which formed a γ-trace arc upon immunoelectrophoresis with anti-CSF was the submaxillary gland (Fig. 3).

Incorporation of C^{14}-amino acid into β-trace was observed with only a few tissues. All the tissues represented in Table II were also examined for β-trace synthesis. Those tissues which occasionally labeled β-trace are shown in Table III; the other tissues were negative. Among the tissues demonstrating β-trace synthesis were fimbriae of the Fallopian tube, testes, vas deferens, and choroid plexus (Table III, Fig. 2).

Discussion. The low sedimentation coefficients obtained from ultracentrifugation studies of both proteins indicate that they are substances of relatively small molecular weights. The higher concentration of these proteins in the CSF than in blood can perhaps be attributed to this property. If it is assumed that the sizes of serum protein molecules influence the rates of entry into CSF, but not the rates of efflux from CSF, a relative accumulation of small molecular weight proteins in CSF may be expected. Recent studies indeed suggest that the concentration of proteins in CSF may be determined mainly by their rates of influx since at least certain proteins, such as albumin, are removed from CSF by bulk absorption(11).

The studies on their sites of formation in the monkey support the contention that these proteins are transported to CSF by blood. It is striking that neither brain tissue, which might have been expected to form these proteins, nor liver, the site of origin of the majority of serum proteins, incorporate labeled amino acid into the trace proteins. γ-Trace is produced by a variety of tissues, among which the submaxillary gland seems to be the most active. This site of origin for γ-trace is confirmed by the finding that submaxillary gland extracts contain a higher concentration of γ-trace than spleen, liver and brain. The only isolated cell type which

TABLE III. *In vitro* Synthesis of β-Trace by Various Rhesus Monkey Tissues.

Tissue	Total No. cultures	Degree of labeling —	w+	+
Testis	1			1
Vas deferens	1		1	
Fimbriae ov	4		2	2
Ovary	8	8		
Choroid plexus	5	3	1	1
Brain	12	11	1	

Degree of labeling was graded from w+ to + according to intensity of autoradiographic image.

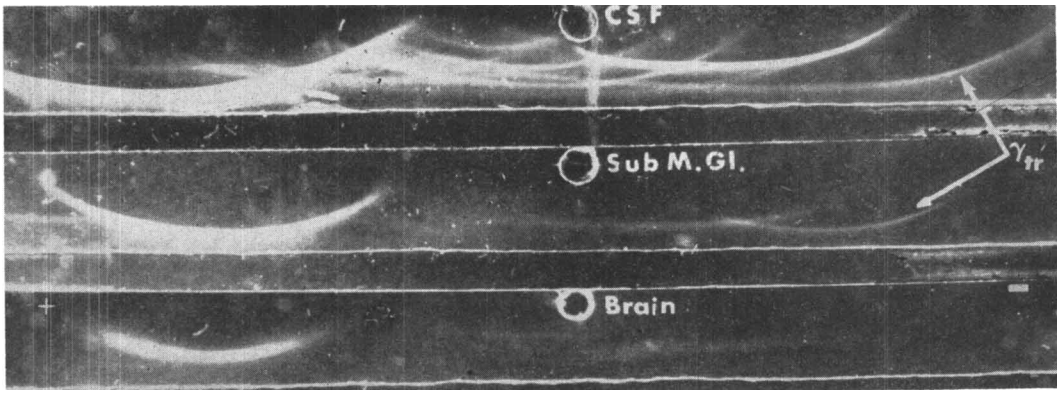

Fig. 3. Immunoelectrophoretic patterns of monkey brain and submaxillary gland extracts as compared to concentrated cerebrospinal fluid (CSF), developed with anti-human CSF. Note the γ-trace arc of the extract of submaxillary gland.

is active in the production of γ-trace is the peritoneal macrophage. Since no functional activity has thus far been ascribed to γ-trace, these sites of origin cannot readily be interpreted at present. Submaxillary gland is a known site of production of nerve growth factor in the mouse(12). The strikingly higher concentration of γ-trace in the CSF from infants as compared to adults(4) suggests a possible relationship between these findings.

Detectable amounts of β-trace are produced by a small number of tissues including testis, fimbriae of the Fallopian tubes, and choroid plexus. The relatively high concentration of this protein in urine and CSF is difficult to explain on this basis.

It should be realized that epithelial cells, which may be involved in the transport of proteins across their cytoplasm, can show labeling of a protein by the technique used in the present study without actually producing this protein. This has, for example, been shown for immune globulin, γA, with mammary gland cultures(13). Such cultures in the presence of C^{14}-amino acid may produce a transport piece which becomes attached to γA, and thus causes labeling of this protein. Studies with the immunofluorescence technique will be needed to determine the cell type responsible for the labeling of the trace protein in various tissues.

Summary. Two isolated trace proteins of beta and gamma mobility from human cerebrospinal fluid were studied to determine their sedimentation coefficients in the ultracentrifuge and their sites of formation. Two sedimentation peaks were obtained for β-trace, 0.5 S and 2.1 S, and one for γ-trace, 1.6 S. The two values for β-trace were thought to be due to polymerization. Production of γ-trace *in vitro* occurred in a number of Rhesus monkey tissues, but was most striking in the submaxillary gland where the protein was present in extractable amounts. A few cultures of choroid plexus, fimbriae of the Fallopian tube, and testis showed C^{14}-amino acid incorporation into β-trace.

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Chemical, Histologic and Immunologic Responses in Rats to CCl_4 II. Relative Influence of Choline Deficiency.* (31898)

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Previous reports have indicated that 2 types of immunoglobulins appear in the blood of rats following carbon tetrachloride (CCl_4) poisoning: those which are not organ-specific, released by hepatocytes within a few hours after exposure and persist for one week; and, the anti-liver antibodies which appear two weeks following exposure, remain for several months and occasionally cross-react with kidney antigens(1,2). Previous work suggested a relationship between these latter antibodies and the liver lipids and liver hydroxyproline levels in non-cirrhotic rats(3). This study reports the results of immunologic assay for isologous anti-liver antibodies and chemical analyses of the livers of rats which had been fed a choline deficient diet, poisoned by CCl_4 or both.

Methods. Male albino rats of the Sprague-Dawley strain weighing 350 g or more were used throughout. Rats were divided into 4 groups: normal controls fed a regular diet, those fed a choline deficient diet 2 or 4 weeks prior to CCl_4 exposure and those given a regular diet but exposed to CCl_4. All food and water was supplied *ad libitum*. The Standard Rockland Rat Diet was used for the regular diet while the choline deficient diet was prepared by Nutritional Biochemical Co. Inhalation doses of CCl_4 were calculated from average minute respiratory volumes, with the assumption that 50% of the CCl_4 was retained. The drug was administered at 6000 ppm using a dual syringe feeder and a stainless steel inhalation chamber equipped with an air flow greater than 20 liters per minute. Rats were sacrificed by decapitation 14 days after their CCl_4 exposure, while the unexposed animals were killed at comparable intervals.

Part of the tissue was quick-frozen, stored at −10°C and the remainder fixed in 10% formalin. Tissue sections for microscopy were stained with Hematoxylin-Eosin.

Liver lipids were measured by the Folch method(4). Liver hydroxyproline was determined by the technique of Leach(5).

Boyden's tanned erythrocyte hemagglutination test was adapted for the Takatsy-type microtiter technique(6). Ouchterlony gel precipitation tests were performed using the Feinberg method(7). Intracutaneous skin tests using liver extracts were read at 20 minutes, 24 and 48 hours. Liver antigens were prepared by extracting the minced tissues with 0.1 M phosphate buffered isotonic saline pH 6.5. Four kinds of liver were extracted: Normal rat liver; and liver from rats given a single 0.25 ml subcutaneous injection of CCl_4, 4 hours, 24 hours or 5 days before.

Results. Table I shows the content of lipids and hydroxyproline in the livers of rats by experimental groups. These data were analyzed for variance by arranging the experimental groups into columns and rows according to the scheme in Table II.

Groups 9, 5 and 6 were significantly different at the 0.5% level for liver content of lipids and hydroxyproline and the lipid/hydroxyproline ratio (L/OHP) displaying in-

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