able mortality. On the other hand, mouse blood and mouse tissues appear to be relatively toxic to neonatal rabbits. On the basis of the reported literature, it appears that the maximum amount of mouse blood or mouse tissue homogenate which can safely be given to neonatal rabbits in the first 14-21 days of life is about 2 ml(4,5). We have observed much the same thing, since our attempts to give more than 2 ml of mouse blood to neonatal rabbits resulted in close to 100% mortality and administration of 1.5 ml to the neonatal rabbits produced about 50% mortality.

Since this study has demonstrated the feasibility of producing in neonatal rabbits a substantial degree of tolerance to the antigens in human blood, this procedure may be found useful in solving other immunologic problems, such as the elucidation of the antigenic relationships in cancer, and other conditions in which presence of an abnormal antigen is suspected.

Conclusion. In neonatal rabbits, a substantial degree of immunologic tolerance has been produced to all the antigens in normal human serum.

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In vitro Degradation of I^{131} Labeled Angiotensin II by Normotensive and Hypertensive Human Serum.* (27187)

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The rate of degradation of intravenously administered angiotensin II-I^{131} has recently been demonstrated to be slower in patients with untreated primary benign hypertension than in normotensive subjects(1-5). The angiotensin II-I^{131} degradation rate, however, in 2 patients with secondary renal hypertension was in the hypertensive range and in one patient was in the normotensive range(2). Biological assays of the blood of hypertensive and normotensive patients indicated that greater quantities of angiotensin were present in the former than in the latter group of patients(6-8). These observations prompted investigation of the effect of normotensive, untreated primary benign hypertensive and untreated secondary renal hypertensive human sera on the degradation of angiotensin II-I^{131} *in vitro*.

Materials and methods. Iodination of angiotensin II (val-5-angiotensin II and ileu-5-angiotensin II)†‡ was accomplished by the Newerly modification of the method of Pressman and Eisen(2). Complete removal of the iodide and iodate remaining after iodination was effected by passage through resin columns.

Sera were obtained from hospitalized normotensive ward patients and from untreated primary benign hypertensive and untreated

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† The val-5-angiotensin II was supplied by Dr. W. E. Wagner, Ciba Pharmaceutical Co., Summit, N. J. and Drs. I. H. Page and F. M. Bumpus, Cleveland Clinic, Cleveland, Ohio furnished the ileu-5-angiotensin II.

‡ Several lots of angiotensin II-I^{131} were supplied through the courtesy of Drs. B. T. Eberle and H. J. Glenn, Abbott Laboratories.

secondary renal hypertensive out-patients. All sera were kept frozen until used.

The technic depends on the ability of human sera to degrade angiotensin II-I^{131} and on quantitative determination of the radioactive degradation-product (iodine-131) by means of paper radiochromato-electrophoresis. Mixtures of 50 μl of human serum and approximately 0.003 μg of angiotensin II-I^{131} were refrigerated for 24 hours at 4°C. Specific activity of the angiotensin II-I^{131} preparations varied between 100 and 250 mc per mg.

The mixtures were then analyzed by paper radiochromato-electrophoresis employing Whatman 3 MM filter paper, 37 by 500 mm and barbital buffer, ionic strength 0.1, pH 8.6. The proteins on the radiochromato-electrophoretic strip were stained with brom phenol blue. The material was applied at the cathode end of the paper strips with the power supply set at 600 volts and the cover of the apparatus kept open. Fluid from the buffer vessels moved toward the center of the paper strips by hydrodynamic forces and the material to be analyzed which was applied at the cathode end of the strips migrated towards the anode. Radiochromato-electrophoresis was employed for approximately 1.5 hours after which the strips were dried in an oven at 110°C for 50 minutes. The strips were assayed for radioactivity in an automatic strip scanner using a thin window gas flow counter with a sensitivity of 0.6×10^6 counts per minute per μc I^{131} above a background of 16 counts per minute. Many of the strips were assayed with a modification of this apparatus employing a linear ratemeter with an integrating count circuit. This device simultaneously registered both the instantaneous count rate and total number of counts on a dual channel recorder.

Separation of angiotensin II-I^{131} from the radioactive degradation-product of angiotensin II-I^{131} (iodine-131) was accomplished by radiochromato-electrophoresis(1,2). Radiochromato-electrophoresis of mixtures of human serum and angiotensin II-I^{131} demonstrated 2 separate peaks of radioactivity; one with the characteristic mobility of angiotensin II-I^{131} and the other with the mobility of iodine-131. The amount of angiotensin II-I^{131} and iodine-131 present was determined either by planimetric measurement of the areas beneath the 2 peaks or from the integrating count circuit of the linear ratemeter. The per cent or fraction of angiotensin II-I^{131} degraded was then calculated.

When tracer quantities of angiotensin II-I^{131} were analyzed by paper radiochromato-electrophoresis the distribution of all of the radioactivity was represented by a smooth, symmetrical curve without skewing or distortion (Fig. 1). An occasional batch of angiotensin II-I^{131} contained a small amount of nonpeptide bound iodine-131 which never was greater than 5% of total radioactivity. When present this small nonpeptide bound iodine-131 fraction necessitated a small correction in calculation of the per cent of angiotensin II-I^{131} degraded by the test serum.

Several experiments were performed in which the amount of angiotensin II-I^{131} varied between 0.0002 μg and 0.3 μg. In general there was no apparent variation in the greater percentage of angiotensin II-I^{131} that was degraded by sera from the hypertensive than from the normotensive patients with these amounts of angiotensin II-I^{131}, although in a few experiments the per cent angiotensin II-I^{131} degraded by sera from the hypertensive patients was slightly increased when 0.003 μg quantities of angiotensin II-I^{131} were employed.

When varying amounts of either normotensive or untreated primary benign hypertensive human sera varying from 0.1 to 100 μl were refrigerated with angiotensin II-I^{131} there was no evidence of any angiotensin II-I^{131} degradation with volumes less than 1.0 μl. The maximum amount of angiotensin II-I^{131} degraded was achieved by approximately 50 μl of human serum. Accordingly this volume was selected for routine use.

Reaction of normotensive and untreated primary benign hypertensive sera with angiotensin II-I^{131} for periods from 1 and 4 days at 4°C indicated that although a slightly greater percentage of angiotensin II-I^{131} was degraded with increasing time of refrigeration there was no increase in the difference between the means of the per cent angiotensin

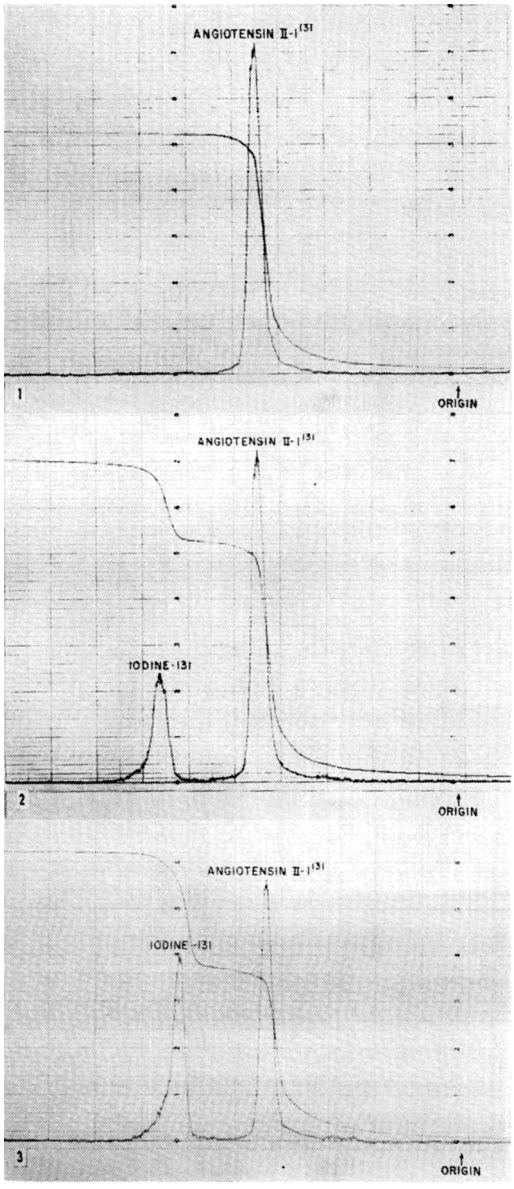

FIG. 1. Paper radiochromatogram of representative preparation of angiotensin II-I^{131} used in these experiments.

FIG. 2. Paper radiochromatogram of same preparation of angiotensin II-I^{131} after reaction with normotensive serum. Amount of angiotensin II-I^{131} degraded is 20%.

FIG. 3. Paper radiochromatogram of same preparation of angiotensin II-I^{131} after reaction with serum from untreated primary benign hypertensive subject. 38% of the angiotensin II-I^{131} is degraded.

II-I^{131} degraded in hypertensive and normotensive groups. Refrigeration of the serum and angiotensin II-I^{131} mixtures for 24 hours was elected as a convenient time that allows the best separation of the per cent angiotensin II-I^{131} degraded between these 2 groups.

Results. When mixtures of angiotensin II-I^{131} and human serum from either normotensive or untreated primary benign hypertensive patients were studied by radiochromato-electrophoresis 2 peaks of radioactivity were present. One peak represented angiotensin II-I^{131}, the other peak represented iodine-131. The untreated primary benign hypertensive sera typically degraded a greater quantity of angiotensin II-I^{131} than did the normotensive sera; hence, a larger fraction of the total radioactivity was present in the iodine-131 peak in these patients on the radiochromato-electrophoretogram (Fig. 2, 3).

The per cent angiotensin II-I^{131} degraded in 14 experiments employing varying numbers of normotensive and untreated primary benign hypertensive sera is presented in Table I. Ninety-three normotensive and 68 untreated primary benign hypertensive determinations were performed. In several sequential experiments the same lot of angiotensin II-I^{131} was employed. Although the means for the per cent angiotensin II-I^{131} degraded varied with different batches, the results indicated that a significantly greater per cent of angiotensin II-I^{131} was degraded by the hypertensive sera than by the normotensive sera. Furthermore, the number of overlapping cases in any individual experiment was almost negligible. Table II is a summary of statistics of the normotensive and untreated primary benign hypertensive groups and indicates that the results are highly significant ($P<10^{-4}$). These investigations indicate that untreated primary benign hypertensive sera degrade a greater quantity of angiotensin II-I^{131} than does serum from normotensive subjects.

Heat inactivation of the normotensive or untreated primary benign hypertensive sera for 30 minutes at 57°C increased the per cent angiotensin II-I^{131} degraded and frozen sera which had been thawed and refrozen before use tended to decrease the per cent angiotensin II-I^{131} degraded. Patients with primary benign hypertension treated with a variety of antihypertensive drugs (Rauwolfia ser-

TABLE I. Per Cent of 0.003 μg Angiotensin II-I^{131} Degraded with 50 μl Sera from Normotensive, Untreated and Treated Primary Benign Hypertensive Subjects.

Exp.	Diagnosis	No. of cases	Range	Mean	Diff. between means of normotensive and untreated hypertensive subjects	No. of overlapping cases of normotensive and untreated hypertensive subjects
1.	A. Normotensive	5	24–40	27	48	0
	B. Untreated hypertensive	4	40–99	75		
	C. Treated "	1		29		
2.	A. Normotensive	8	14–22	17	17	1
	B. Untreated hypertensive	5	17–41	34		
	C. Treated "	4	12–29	21		
3.	A. Normotensive	8	21–36	29	20	1
	B. Untreated hypertensive	5	31–58	49		
	C. Treated "	4	23–36	31		
4.	A. Normotensive	4	47–57	52	36	0
	B. Untreated hypertensive	4	83–99	88		
	C. Treated "	6	55–85	69		
5.	A. Normotensive	4	27–35	31	7	1
	B. Untreated hypertensive	4	31–48	38		
	C. Treated "	3	15–21	17		
6.	A. Normotensive	4	35–41	38	25	0
	B. Untreated hypertensive	5	51–71	63		
	C. Treated "	7	52–77	60		
7.	A. Normotensive	7	18–30	24	9	1
	B. Untreated hypertensive	5	28–50	33		
	C. Treated "	6	20–29	26		
8.	A. Normotensive	7	20–27	24	5	1
	B. Untreated hypertensive	5	25–35	29		
	C. Treated "	6	20–29	26		
9.	A. Normotensive	4	12–32	27	0	1
	B. Untreated hypertensive	3	16–40	27		
	C. Treated "	8	11–56	26		
10.	A. Normotensive	15	6–22	15	13	0
	B. Untreated hypertensive	4	23–33	28		
	C. Treated "	12	11–22	17		
11.	A. Normotensive	5	10–21	21	3	1
	B. Untreated hypertensive	4	14–32	24		
	C. Treated "	3	15–21	17		
12.	A. Normotensive	6	17–30	24	3	2
	B. Untreated hypertensive	5	24–35	27		
	C. Treated "	5	13–26	18		
13.	A. Normotensive	6	5–13	9	4	1
	B. Untreated hypertensive	5	8–16	13		
	C. Treated "	5	4–13	8		
14.	A. Normotensive	10	12–26	18	3	2
	B. Untreated hypertensive	10	20–31	21		
	C. Treated "	9	10–20	18		

pentina derivatives, oral diuretics, ganglion-blocking agents and guanethidine), either singly or in combination, and with partial or complete reduction of blood pressure values to normal generally had determinations of per cent angiotensin II-I^{131} degraded that roughly paralleled their hypertensive status at the time the determination was made.

Table III shows the results in untreated and treated primary benign hypertensive subjects and indicates that the differences in the per cent angiotensin II-I^{131} degraded are significant ($P<10^{-2}$). Reaction of angiotensin II-I^{131} with 20 μl of human serum albumin usually resulted in degradation of almost all of the angiotensin II-I^{131}. In 2 cases of

TABLE II. Summary of Statistics for Comparison of Per Cent of 0.003 μg Angiotensin II-I^{131} Degraded with 50 μl Sera from Normotensive and Untreated Primary Benign Hypertensive Subjects.

Type of case	No. of cases	Mean & stand. dev.
Normotensive	93	23 ± 11.1
Untreated hypertensive	68	37 ± 22.0
S.E. of difference = 2.64		$P < 10^{-4}$

secondary renal hypertension the per cent angiotensin II-I^{131} degraded was within the normal range.

Discussion. It is apparent from these results that untreated primary benign hypertensive sera enhance the degradation of angiotensin II-I^{131} *in vitro*. This may be due to the presence of one or more unique factors in the sera of these patients or to the increased concentration or biologic activity of a factor (angiotensinase) normally present in human sera. Whether this factor (or factors) is present in all of the serum fractions is being investigated. The observation that heat inactivation of serum, whether from a normotensive or untreated primary benign hypertensive subject, increases the amount of angiotensin II-I^{131} degraded suggests that an inhibitor may be present in human serum which restrains the action of this factor (or factors) and that this inhibitor is destroyed by heating to 57 °C for 30 minutes. Additional studies have been designed to define the properties of this inhibitor.

The reduction in the per cent angiotensin II-I^{131} degraded in the treated primary benign hypertensive patients whose blood pressures were normal or almost normal implies that the factor or factors which enhance the degradation of angiotensin II-I^{131} are decreased or altered by the antihypertensive treatment. The greater *in vitro* degradation of angiotensin II-I^{131} by untreated primary benign hypertensive sera than by normotensive sera is in contrast to both the slower turnover *in vivo* of angiotensin II-I^{131}(1-5) and the greater hypertensive response following intravenous administration of incubated angiotensin II(9) in hypertensive than in normotensive subjects. This implies that factors are present in the hypertensive patient which result in a slow *in vivo* turnover rate despite a relatively great *in vitro* degradation.

TABLE III. Summary of Statistics for Comparison of Per Cent of 0.003 μg Angiotensin II-I^{131} Degraded with 50 μl Sera from Untreated and Treated Primary Benign Hypertensive Subjects.

Type of case	No. of cases	Mean & stand. dev.
Untreated hypertension	68	37 ± 22.0
Treated "	79	78 ± 18.5
S.E. of difference = 3.33		$P < 10^{-2}$

The normal values for the per cent angiotensin II-I^{131} degraded in the 2 patients with secondary renal hypertension are in contrast to the results obtained in the untreated primary benign hypertensive group. If this difference is consistent for a larger series, it may be feasible to employ this procedure to distinguish patients with secondary renal hypertension from those with primary hypertension.

Summary. Refrigeration of angiotensin II-I^{131} *in vitro* with human sera from normotensive, untreated primary benign hypertensive and secondary renal hypertensive subjects demonstrated that greater quantities of angiotensin II-I^{131} were degraded by the untreated primary benign hypertensive patients than by the normotensive or secondary renal hypertensive patients. Evidence for presence of a serum factor (or factors) enhancing angiotensin II-I^{131} degradation and for a heat labile inhibitor has been advanced. The greater *in vitro* degradation of angiotensin II-I^{131} by untreated primary benign hypertensive patients than by normotensive subjects contrasts with the *in vivo* turnover rates.

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Effect of Vegetable Oils on Plasma Lipids of Rhesus Monkeys.* (27188)

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The effect of dietary fats on serum cholesterol has been the subject of many recent investigations. Ahrens(1) reviewed the contributions of different workers, including his own group, which showed that the concentration of serum cholesterol in man is influenced by the nature of the dietary fat. Connor *et al.*(2) recently reported that dietary cholesterol is one of the important factors which influence the blood cholesterol level in human beings. In experiments with rats Swell and Flick(3) observed that oleic acid feeding resulted in higher blood cholesterol levels than did stearic acid. Lin *et al.*(4,5) also observed increased fecal excretion of cholesterol when the animals were fed palmitic acid or stearic acid. Aftergood *et al.*(6) observed that diets based on lard resulted in higher serum cholesterol than did diets based on cottonseed oil. Portman *et al.*(7) found in Cebus monkeys, that those fats which were most rich in polyunsaturated fatty acids tended to promote the lowest serum choleserol level. Sunflowerseed oil, an unsaturated fat, lowers serum cholesterol while hydrogenated coconut oil produces a sustained rise of serum cholesterol (8). These studies indicated a possible inverse relationship between iodine number of dietary fat and serum cholesterol. Jones *et al.*(9) observed, in cholesterol fed chicks, a cholesterol depressing effect of corn oil, but showed that this effect could not be explained by the fatty acid component of the oil. Experimental data on other animals including man also did not prove the hypothesis that degree of unsaturation and essential fatty acid composition of dietary fat controlled the serum cholesterol level(4,10,11). The differences between animal species complicate the picture. More information is needed regarding the properties of dietary fat and the effect of their prolonged administration on serum cholesterol level.

The present work deals with the effects of feeding 3 edible oils commonly used in India, mustard oil, coconut oil and sesame oil, on the different fractions of plasma lipids in rhesus monkeys.

Materials and methods. Eleven rhesus monkeys were fed a basal diet consisting of wheat flour 64 parts, ground Bengal gram (*Cicer arietinum*) 20 parts, commercial casein 12 parts, calcium carbonate 3 parts and common salt 1 part. The diet contained 2% fat, 19% protein, 65% carbohydrate and 1% fiber. Cholesterol of the diet was extracted with petroleum ether in a soxhlet apparatus, petroleum ether removed, the residue extracted with acetone, alcohol was added, hydrolysed with a drop of 33% potassium hydroxide, cholesterol precipitated with digitonin and estimated by the method of Sobel and Meyer(12). Cholesterol of the diet was 15 mg per 100 g possibly derived from crude casein. The animals were fed in addition 50 mg ascorbic acid per animal per day and 5 drops of a concentrate of Vit. A and D twice a week. The animals grew normally and consumed 150 g of the diet per day. Average weight of the animals was 4 kg.

Blood was collected by vein-puncture on the morning after an over night fast, for analysis of the different fractions of plasma lipids. The animals were then divided into 5 groups and were fed basal diet only, basal diet with 3 g cholesterol mixed with the diet,

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