junctiva cells or in the allantoic sac of the chicken embryo. This is uncommon among myxoviruses. It represents a distinct advantage since in the egg the virus can be cultivated in large quantity with ease, and then the virus can be used for experiments in human cells. A somewhat surprising aspect of this flexibility of the adapted virus is the finding that, whether the virus is grown in conjunctiva cells or in the egg, the conjunctiva cell monolayer is considerably more sensitive in detecting infectious virus particles than is the egg.

Summary. Sendai strain of parainfluenza 1 virus previously passed in embryonated eggs was found to be adaptable to the stable line of human conjunctiva cells. Evidence for its multiplication in these cells was based on its capacity to produce typical CPE in 5-7 days throughout 17 serial passages, on its ability to initiate macroscopic plaque formation and on the nature of its single step growth cycle. The adapted virus could be cultivated in either conjunctiva cells or chick embryos. The former was more sensitive to viral infection (ratios of PFU:EIU up to 27) whereas higher virus titers were obtained from the latter.

1. Matsumoto, M., Am. Rev. Resp. Dis., 1963, v88, 46.
2. Chanock, R. M., Parrott, R. H., Johnson, K. M., Kapikan, A. Z., Bell, J. A., ibid., 1963, v88, 152.
3. Jensen, K. E., Minuse, E., Ackermann, W. W., J. Immunol., 1955, v75, 71.
4. Shimizu, T., Ishizaki, R., Kono, Y., Kumagai, T., Arai, S., Sasahara, J., Ishii, S., Matumoto, M., Japan J. Exp. Med., 1955, v25, 211.
5. Bukrinskaya, A. G., Acta Virol., 1958, v2, 208.
6. Heath, R. B., Tyrrell, D. A. J., Arch. Virusforsch., 1959, v8, 577.
7. Mannweiler, E., ibid., 1961, v10, 647.
8. Matsumoto, T., Maeno, K., Japan. J. Microbiol., 1961, v5, 89.
9. Zhdanov, V. M., Bukrinskaya, A. G., Prob. in Virol., 1960, v5, 245.
10. Zhdanov, V. M., Bukrinskaya, A. G., Azaslova, N. B., J. Immunol., 1961, v81, 647.
11. Gresser, I., Proc. Soc. Exp. Biol. and Med., 1961, v108, 303.
12. Bukrinskaya, A. G., Zhdanov, V. M., Nature, 1963, v200, 920.
13. Henle, W., Henle, G., Deinhardt, F., Bergs, V. V., J. Exp. Med., 1959, v110, 525.
14. Ho, Y. D., Gorbunova, A. S., Acta Virol., 1962, v6, 193.
15. Ishida, N., Homma, M., Virology, 1961, v14, 486.
16. Demont, G., Berkaloff, A., Colobert, L., Compt. Rend., 1962, v255, 421.
17. Chang, R. S. M., Proc. Soc. Exp. Biol. and Med., 1954, v87, 440.
18. Eagle, H., Science, 1955, v122, 501.
19. Reed, L. J., Muench, H., Am. J. Hyg., 1938, v27, 493.
20. Granoff, A., Virology, 1955, v1, 516.
21. Chanock, R. M., Parrott, R. H., Cook, K., Andrews, B. E., Bell, J. A., Reichelderfer, T., Kapikan, A. Z., Mastrota, T. M., Huebner, R. J., New Eng. J. Med., 1958, v258, 207.
22. Henle, W., Deinhardt, F., Henle, G., Internat. Cong. for Microbiol., Stockholm, 1958, 7th, 268.
23. Matsumoto, T., J. Immunol., 1961, v87, 590.
24. Isaacs, A., Edney, M., Aust. J. Exp. Biol. Med. Sci., 1950, v28, 635.

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In vitro Production of Coagulation Factors VII-X by Liver Slices from Rats Fed Atherogenic Diets.* (29944)

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Previous reports from this and other laboratories indicate that rats fed atherogenic diets develop high levels of many blood coagulation factors (1-5) including Factor VII-X. Theoretically, the elevated levels could result from increased synthesis, diminished degradation, or both. We have therefore meas-

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ured Factor VII-X production by liver slices incubated *in vitro*(6,7) in an endeavor to assess synthesis. Our results are compatible with the hypothesis that excessive production is one of the mechanisms responsible for the high level of Factor VII-X found in the blood of these animals.

Materials and methods. Thirty-six male CFN rats weighing 308-433 g were paired by weight, placed in separate cages and fed either control or fat-cholesterol (test) diet previously described(5). The latter diet contained 40% beef fat, 5% cholesterol, 2% cholic acid, and 0.3% thiouracil. After 19-34 days on the diet an animal from each group was anesthetized with Pentobarbital Sodium† and the liver perfused with buffer as described by Pool and coworkers(6,7) after which liver slices of 0.25-0.35 mm thickness were prepared with a tissue slicer.‡ Three to 8 flasks, each containing approximately 300 mg wet weight of liver slices in 5 ml bicarbonate buffer were incubated at 38°C in a Dübnoff shaker after gassing with 95% O_2-5% CO_2. A second set of flasks from the same liver was shaken at 0°C and served as a blank. After incubation for 1 to 4 hours, 1 ml of 2% EDTA was added and the contents of each flask homogenized. Aliquots of each flask were tested for Factor VII-X by the method of Owren and Aas(8), pooled normal rat plasma serving as a standard containing 100 units per ml. In addition a trichloracetic acid (TCA) precipitate was prepared by the method of Burton(9). This involved sequential washing of the TCA precipitate with dilute TCA, 95% ethanol and ethanol-ether (3:1). The precipitates were then extracted with hot 5% TCA, the protein residue dissolved in 10% NaOH, and protein measured by the Folin-Ciocalteau method(10). This extraction procedure yields values lower than in the total homogenate which is incompletely soluble in the alkali. Net Factor VII-X synthesis was calculated by subtracting the mean content found in flasks kept at 0°C from those incubated at 38°C. The net Factor VII-X production(6) was expressed in 2 ways: (1) per unit weight protein, (2) per unit wet weight of liver in the flask. The experiment was performed in 2 series some months apart. The first series consisted of 8 pairs of rats and the second series 10 pairs.

† Nembutal® Sodium (Abbott Laboratories, Chicago, Ill.).

‡ #140, Harvard Apparatus Co., Inc., Dover, Mass.

TABLE I.

	Mean liver wt		Liver protein (mg) per 100 mg wet wt of liver
	Total wt (g)	Per 100 g carcass wt	
Control	15.48† (1.91)	3.73† (.32)	4.42* (.93)
Test	12.97 (2.36)	5.00 (.76)	3.63 (1.06)

Mean liver weight and protein content of liver from control and test animals. Figures in parentheses represent ± standard deviation. The significance of the differences between the groups is shown thus: * $p = .02$ † $p < .01$.

Results. Elevated plasma Factor VII-X levels were found in all 18 rats on the test diet (150% ± 31.0 S.D.) compared with control (100% ± 20.2 S.D.). The difference is significant ($p<0.01$).

Test animals lost weight and their livers appeared fatty. Liver weight and protein content of the livers of the 2 groups of rats are shown in Table I. The livers of test rats contained less protein per unit wet weight than did control rat livers.

More Factor VII-X per mg protein was produced in the flasks of liver slices from test rats in 15 out of the 18 paired experiments. In the remaining 3 paired experiments control exceeded test, but in 2 of these experiments very little Factor VII-X was produced in any of the flasks (Fig. 1, 2) and in the 3rd the differences were minimal. Statistical analysis (Mann Whitney U Test) of all 18 pairs showed that significantly more Factor VII-X was produced in the flasks from test rats ($p<0.02$).

The results were similar but not as marked ($p = 0.05$) when the Factor VII-X present in the flasks was expressed per unit wet weight of liver in the flask, since control liver contained more protein per unit wet weight. The ranges of Factor VII-X production (and the median values) expressed per mg protein in the flask and per gram wet weight of liver in the flask are shown in Table II.

Total Factor VII-X production by the

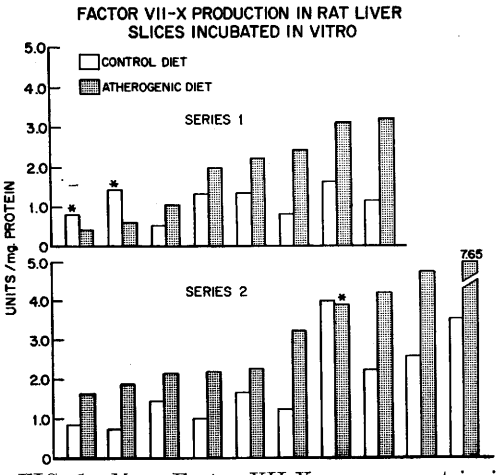

FIG. 1. More Factor VII-X per mg protein is produced in flasks derived from test animals in 15 out of 18 paired experiments. The 3 exceptions are starred. The figure represents production after 4 hr incubation.

whole liver was calculated from total liver weight, liver protein and Factor VII-X production per unit weight liver protein. In series I test rat livers produced 964 units (control 797) and in series II 1403 units (control 970). These differences were not significant but for series II $0.05<p<0.10$.

There was considerable variation in amount of Factor VII-X production by liver slices from one pair of rats to another and from one series to the other. The variations could not be accounted for by alterations in technic which eliminated drying of slices prior to weighing, by differences of sharpness of tissue slicer blade, by elimination of shaking in blank flasks or by addition of EDTA to blank flasks prior to addition of liver slices.

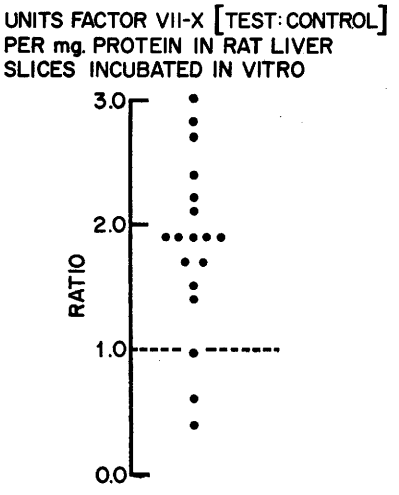

FIG. 2. Significantly more Factor VII-X per mg protein is produced in flasks derived from test rat livers. Median value for ratio (test:control) is 1.9.

Discussion. These findings indicate that larger amounts of Factor VII-X are produced in incubated flasks of liver slices derived from test animals than from control rats. The assay system used is sensitive to the presence of both Factor VII and Factor X but the increase noted during incubation reflects mainly increase in Factor VII(6). Pilot observations revealed that the amount produced in flasks after one hour's incubation was higher than that in the blanks but significantly less than that produced after 4 hours' incubation; this suggests active synthesis. Results were similar when expressed per mg of protein or per unit wet weight of liver present in the flask. At 4 hours, the flasks from test animals produced 1.9 times (median) as much activity as control flasks. It is conceivable that some of the increased content of Factor VII-X was due to diminished degradation.

TABLE II. Units Factor VII-X Production.

	Per mg protein in flask		Per g wet wt liver in flask	
	Control	Test	Control	Test
Series 1	.55–1.61 (1.18)	.45–3.24 (2.12)	33– 72 (47)	17–124 (74)
Series 2	.75–4.01 (1.55)	1.68–7.65 (2.75)	36–167 (60)	51–253 (110)

Figures given show range; figures in parentheses indicate median value. Figures represent production after 4 hr incubation.

Calculations made of total Factor VII-X production by the liver did not reveal significant differences between test and control rats. Test rats did not produce less despite the smaller size of the animal, the liver, and its blood volume(11); the tendency, if any, was towards greater production.

It was not possible to relate Factor VII-X production directly to liver/carcass weight ratio or plasma level of Factor VII-X. Finally the marked variation which occurred during each series and from one series to the other remains unexplained.

Conclusion and summary. Coagulation Fac-

tor VII-X production by liver slices was studied because it was found that plasma Factor VII-X levels were significantly elevated in rats fed atherogenic diets. Although unexplained variations occurred, in 15 out of 18 paired experiments the production of Factor VII-X during incubation was greater in flasks containing liver slices from test animals than from controls. Expressed as a ratio (test:control) the median value of Factor VII-X production per mg protein in the flask was 1.9. Thus there appeared to be increased synthesis of Factor VII-X in flasks from test rats though the possibility of delayed degradation was not excluded.

1. Davidson, E., Howard, A. N., Gresham, G. A., Brit. J. Exp. Path., 1961, v42, 195.

2. Kudrjashov, B. A., Bazasian, G. G., Sytina, N. P., Andreenko, G. V., Nature (London), 1961, v189, 67.

3. Fisher, L. M., Kagan, E., Kupfer, H. G., Circ. Res., 1963, v13, 529.

4. Naimi, S., Goldstein, R., Notham, M. M., Wilgram, G. F., Proger, S., J. Clin. Invest., 1962, v41, 1708.

5. Merskey, C., Wohl, H., Thromb. Diath. Haemorrh., 1964, v10, 295.

6. Pool, J. G., Robinson, J., Am. J. Physiol., 1959, v196, 423.

7. Pool, J. G., Borchgrevink, C. F., ibid., 1964, v206, 229.

8. Owren, P. A., Aas, K., Scand. J. Clin. Lab. Invest., 1951, v3, 201.

9. Burton, K., Biochem. J., 1956, v62, 315.

10. Ratnoff, O. D., Menzie, C., in The Coagulation of Blood: Methods of Study, Ed., Tocantins, L. M., Grune & Stratton, N. Y., 1955, p155.

11. Wohl, H., Merskey, C., Am. J. Physiol., 1964, v206, 762.

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Effects of Triparanol on the Hyperlipemia of Experimental Nephrosis.* (29945)

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We(1-5) have repeatedly observed and the literature convincingly demonstrates that hyperlipemia and hypercholesteremia of varying degrees develop along with hypoproteinemia during the development of nephrosis. This is true both of clinical nephrosis in patients and of nephrosis produced experimentally in dogs and rats by administration of antikidney serum. This inverse relationship between the occurrence of hypoproteinemia and the occurrence of hyperlipemia was so consistent that we decided to study the effects of an antilipemic agent, triparanol (MER-29), which is known to arrest cholesterol synthesis at the desmosterol stage. We wondered whether arrest of cholesterol synthesis and depression of the hyperlipemia would have any effect on the loss of plasma proteins and the resulting hypoproteinemia.

Background information. A survey of the literature revealed elaborate studies on the relationship of hypoproteinemia and hyperlipemia in nephrosis without any definite consensus to pinpoint that relationship etiologically or consequentially.

The nephrotic syndrome, whether experimentally produced or clinically encountered, is not a disease but a complex of clinical signs and symptoms and a definite set of chemical findings in the blood and urine that are sometimes encountered in other disease entities. The salient features characterizing this syndrome are massive proteinuria, hypoproteinemia, hypoalbuminemia, hyperlipemia, hypercholesteremia and usually edema.

The site of the pathologic changes in the nephrotic syndrome is the kidney generally and the glomerular basement membrane specifically. Thickening of the glomerular base-

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