been isolated from milk[13] and that the reported solubility properties of this substance are roughly those of the inhibitor. The relation between the milk inhibitor and lactomucin is being investigated.

Summary. Milk is capable of inhibiting hemagglutination by influenza viruses, the inhibitory effect being greater against heated than against unheated viruses. The inhibitor, which is non-dialyzable and moderately heat stable, occurs in the whey and can be salted out by half-saturation with ammonium sulfate. Evidence is presented that the inhibitor is a characteristic component of milk rather than a specific antibody.

[13] Sorensen, M., and Sorensen, S. P. L., *Compt. Rend. Trav. Lab. Carlsberg, Sér. Chim.*, 1938-41, **23**, 55.

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Effect of Dicumarol on Concentration of the Labile Factor.* (17390)

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The observation that the loss of prothrombin activity in stored human plasma could be restored by adding to it rabbit or dog plasma which was markedly depleted of prothrombin by means of dicumarol, led to the discovery of the labile factor and to the assumption that this agent is not affected by dicumarol.[1] The development of a quantitative procedure for estimating the labile factor in blood,[2] now makes it possible to test the validity of this assumption.

Experimental. Rabbits and dogs were given dicumarol orally (5 mg per kilo body weight daily) until the prothrombin time became markedly prolonged. The prothrombin activity was determined by the original one-stage procedure.[3] For the estimation of the concentration of the labile factor, the method of Quick and Stefanini[2] was employed. The method consists essentially in determining the prothrombin time of stored plasma after the addition of a measured amount of the plasma to be tested which has been deprothrombinized by adsorption with tricalcium phosphate. The reduction of the prothrombin time is a measure of the concentration of the labile factor.

Results. From the data in Table I it can be seen that the concentration of the labile factor in normal rabbit plasma is remarkably constant. This is also true of dog plasma, but the concentration is only one-fifth that of rabbit plasma.[2] When dicumarol is administered the prothrombin activity as measured by prothrombin time decreases progressively and after one week may reach a level below one per cent of normal (Table II). In spite of this drastic decrease, the concentration of the labile factor shows no greater fluctuation than may be attributed to the inherent variations of the method. It can be concluded that dicumarol does not influence the labile factor in rabbits and dogs. Preliminary studies indicate that this is also true in humans.

Owren,[4] Fantl and Nance[5] and Seegers and Ware[6] agree at least tentatively that their respective agents, factor V, accelerator factor and Ac-globulin are the same as the labile factor. The finding of Fahey, Olwin and Ware[7] that dicumarol causes at least a moderate decrease in Ac-globulin in dogs is not in agreement with the present results. It is likely that the difference in findings can

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[1] Quick, A. J., *Am. J. Physiol.*, 1943, **140**, 212.

[2] Quick, A. J., and Stefanini, M., *J. Lab. Clin. Med.*, 1948, **33**, 819.

[3] Quick, A. J., *J.A.M.A.*, 1938, **110**, 1658.

[4] Owren, P. A., *Biochem. J.*, 1948, **43**, 136.

[5] Fantl, P., and Nance, M. H., *Med. J. Australia*, 1948, **1**, 98.

[6] Seegers, W. H., and Ware, A. G., *Am. J. Clin. Path.*, 1949, **19**, 441.

[7] Fahey, J. L., Olwin, J. N., and Ware, A. G., PROC. SOC. EXP. BIOL. AND MED., 1948. **69**, 491.

TABLE I.
Concentration of the Labile Factor in Normal Rabbit Plasma.*

Stored human plasma, cc	.099	.098
Rabbit deprothrombinized plasma, cc	.001	.002
	Prothrombin time (in sec.)	
Rabbit 1	14	12
" 2	14	12
" 3	13½	12
" 4	14	11
" 5	14½	12
" 6	13½	12
" 7	14½	12½
" 8	13	11
" 9	14	12
" 10	14	12

* The stored human plasma had a prothrombin time of 45 sec. The rabbit plasma was treated with tricalcium phosphate gel (0.012 M) to remove prothrombin.

TABLE II.
Effect of Dicumarol Treatment on Concentration of the Labile Factor in Rabbits and Dogs.

Animal	Day of treatment	Prothrombin time of whole plasma (sec)	Prothrombin time (sec) of mixture: Stored human plasma,† cc / Deprothrombinized plasma of animal treated,‡ cc	.099 / .001	.098 / .002
Rabbit 1*	0	7		13	12
	1	11		13	11½
	2	8½		13½	11
	3	8½		13	11
	4	10		12½	10½
	5	12		12½	11
Rabbit 2*	0	6½		13½	12
	1	13		14	11½
	2	20		13	11
	3	27		13	11
	4	53		13	11
	5	83		13	11
Dog 1	0	6		20	16
	1	11		22	15
	2	17		19	14
	3	25		18	15
	4	39		19	15½
Dog 2	0	6		18	14
	1	12		22	16½
	2	17		20	14½
	3	27		22	16
	4	40		20½	16

* The data were selected to show that the sensitivity to dicumarol bears no relation to variations of the labile factor.

† The stored human plasma had a prothrombin time of 45 sec.

‡ Rabbit and dog plasma were treated with tricalcium phosphate gel (0.012 M) prior to use, to remove prothrombin.

be accounted for by the methods for determining the labile factor (Ac-globulin). With the method of Ware and Seegers[8] the variation of the labile factor in rabbit plasma was found to be from 92 to 310 units and in dogs from 158 to 203 units.[9] In contrast, the method of Quick and Stefanini showed that the labile factor in rabbit plasma was relatively con-

[8] Ware, A. G., and Seegers, W. H., *J. Biol. Chem.*, 1948, **172**, 699.

[9] Murphy, R. C., and Seegers, W. H., *Am. J. Physiol.*, 1948, **154**, 134.

stant, and was 5 times higher than in dog plasma.

These differences in results are probably traceable, at least in part, to technical factors of the methods. It appears that the procedure employed in this study is more sensitive and perhaps subject to less error. Nevertheless, when the two methods are applied to normal plasmas they yield results which roughly parallel each other, and both show a low concentration of labile factor in human blood. More important but far more difficult to harmonize and discuss critically are the differences in views concerning the action of the labile factor. Owren, Fantl and Seegers look upon this factor as an accelerator or catalyst, while the writers conclude that it reacts stoechiometrically since a manifold increase in its concentration does not alter the prothrombin time nor the speed of prothrombin conversion.[10] Recently findings have been made which clearly suggest that prothrombin in human blood exists partly in an active form and partly in a precursor state.[11] This discovery introduces a new element in the clotting mechanism requiring evaluation. It is likely that the concept that prothrombin *per se* can change its convertibility or that catalysts can alter it, will require reinterpretation. Since the one-stage method measures active prothrombin, and the two-stage probably total prothrombin, findings such as those reported by Owen and Bollman[12] that the two methods when applied to dicumarolized blood showed marked difference in prothrombin level can be explained without postulating a factor which changes the rate of conversion of prothrombin to thrombin.

Conclusions. The labile factor of the prothrombin complex is relatively constant in normal rabbit and dog blood. It is not reduced by dicumarol even after the prothrombin activity falls to a very low level.

[10] Quick, A. J., and Stefanini, M., *J. Lab. Clin. Med.*, 1949, **34**, 973.

[11] Quick, A. J., and Stefanini, M., *J. Lab. Clin. Med.*, 1949, **34**, 1203.

[12] Owen, C. A., and Bollman, J. L., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 231.

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Intracellular Bile Canaliculi in the Rabbit Liver. (17391)

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There is no controversy as to the general pattern of the bile canaliculi in the mammalian liver with but one exception. This concerns the existence of intracellular branches.

Gomori's[1] technic for the histochemical demonstration of alkaline phosphatase furnishes a very convenient method for the study of the bile capillaries under normal and abnormal conditions, since the bile canaliculi in the liver of some species show striking staining properties.[2-4] The results obtained with this method in the liver of rabbits under normal conditions and after experimental biliary obstruction, strongly suggest the existence of intracellular branches.

Material and Method. In medium sized rabbit under ether anesthesia, a liver biopsy was taken and the cystic and bile ducts were ligated. Twenty-two animals were allowed to survive from 1 to 31 days. In addition, livers from normal animals were available. Sections from tissues fixed in cold acetone were stained with Gomori's method[1] as modified by Kabat

[1] Gomori, G., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 23.

[2] Gomori, G., *J. Cell. and Comp. Physiol.*, 1941, **17**, 71.

[3] Wachstein, M., and Zak, F. G., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 73.

[4] Jacoby, F., *J. Phys.*, 1947, **106**, 23P.