TABLE I.
Changes in Renal Hemodynamics of Human Subjects Induced by Subcutaneous Injections of 5968 (Ciba).

Diagnosis	Dose (mg)	Before*				After†				Change in C_{PAH}‡
		C_{PAH}	C_T	F.F.	B.P.	C_{PAH}	C_T	F.F.	B.P.	
Ess. hypertension	20	429	128	.30	200/115	593	116	.20	175/70	+38
" "	12	432	86	.20	180/100	628	108	.17	180/100	+45
" "	10	405	71	.17	170/80	555	74	.13	160/55	+36
" "	10	315	84	.27	160/90	530	104	.20	160/80	+68
" "	10	352	118	.34	160/80	469	126	.28	135/65	+33
" "	8	606	98	.16	140/95	850	79	.09	130/90	+40
Chronic nephritis	10	182	32	.18	175/85	233	35	.15	130/70	+28
" "	8	822	125	.15	145/75	950	114	.12	145/17	+16
Normal	9	730	140	.19	125/75	911	169	.19	115/75	+25
"	9	628	104	.17	110/60	884	129	.15	110/50	+41
"	9	733	149	.20	130/95	1167	143	.12	130/80	+59
"	8	720	153	.21	125/80	892	173	.19	125/65	+24

* Each value represents average of 3 control periods.
† Maximal changes observed after injection.
C_{PAH}: Sodium para-aminohippurate plasma clearance (cc/min.).
C_T: Sodium thiosulphate plasma clearance (cc/min.).
F.F: Filtration fraction.
B.P.: Blood pressure (mm of mercury).
Ess. Hypertension: Essential hypertension.
‡ Expressed as per cent of control value.

clearance technic,(1) it became evident that renal hyperemia could be elicited in nearly every subject. A similar effect has also been demonstrated in cats by thermostromuhr measurements.(3)

Our data, presented in Table I, demonstrate that this drug can cause increase in renal plasma flow in man varying from 16 to 68%, averaging 38.6%. The glomerular filtration rate, as reflected by the clearance of thiosulphate, is not consistently affected. The filtration fraction is always decreased. In some cases, urine volumes are markedly increased. Blood pressure shows a definite reduction only in some hypertensive patients. Therefore, the influence of the drug upon the kidney is somewhat similar to that of pyrogenic substances. The responses obtained are moderately prolonged, lasting 1 to 2 hours or more. Toxic manifestations are relatively unimportant. Moderate tachycardia may be observed in some cases. Effects of the drug upon cardiac output are being investigated.

From these results, it can be concluded that the drug 5968 (Ciba) is a renal vasodilator in man.

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Effect of Thiazine, Oxazine and Phenazine Dyes on Normal and Heparinized Rabbit Plasma.* (17592)

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The recent work of Allen and Jacobson(1) showing the usefulness of Toluidine Blue as a heparin inactivator in x-irradiation damage has revived interest in the field of synthetic heparin inactivators. Haley and Stolarsky(2)

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1. Allen, J. G., and Jacobson, L. O., *Science*, 1947, v105, 388.

2. Haley, T. J., and Stolarsky, F., *J. Am. Pharm Assn.*, Sci. Ed., in press.

using MacIntosh's(3) *in vitro* method evaluated 19 dyes and found that only those belonging to the thiazine, oxazine and phenazine series which had a free primary amine group were active heparin inactivators. This type of chemical evaluation, while it determines quantitatively the amount of heparin precipitated and removed from solution, does not give any indication of biological heparin inactivation. Such chemical studies must be supplemented by investigations of the effect of heparin inactivators upon whole blood or plasma *in vitro*. This type of investigation is extremely important because it has been reported that small amounts of Toluidine Blue increase the anticoagulant effect of heparin while larger amounts of the dye decrease coagulation time but do not entirely inactivate heparin.(4,5) Similar results have been reported for Safranine, Neutral Red and Janus Green.(6-8) However, these results do not cover the entire group of effective heparin inactivating dyes nor do they give exact quantitative data. We decided to investigate the effect of our most potent chemical inactivators of heparin(2) upon the *in vitro* coagulation of plasma in an attempt to quantitate the concentration of dye required to return the coagulation time to the normal control level and also determine the overall effect of these dyes upon the coagulation mechanism.

Experimental. Blood was obtained from 6 rabbits by cardiac puncture every 2 weeks. The pooled blood was mixed with 3.8 per cent citrate at a ratio of 1:4, centrifuged and the supernatant plasma removed and stored at 4°C until used. During the preliminary phase of the work any plasma showing fibrin threads was discarded. In the latter phases we found, in confirmation of Lewis and De Maria,(9) that filtration through glass wool removed any fibrin present and gave a plasma with good coagulation properties. A modified Astrup(10) method was used for studying the effect of the various dyes on the coagulation time of normal and heparinized rabbit plasma. The following solutions were employed: Difco Thromboplastin 0.01% in normal saline, calcium chloride ($CaCl_2 \cdot 2H_2O$) 0.68% in distilled water, and powdered heparin sodium Upjohn (120 Toronto Units/mg) 0.1% in normal saline. The majority of the dyes were made up in concentrations of 5 to 200 μg per 0.1 cc. Janus Green gave a precipitate above 25 μg per 0.1 cc making it impossible to study the effect of higher concentrations of this dye. Neutral Red was used up to its maximum solubility, 800 μg per 0.1 cc. Thionine was not soluble above 25 μg per 0.1 cc and Safranine above 100 μg per 0.1 cc. The usual solubilizing procedures, ethanol and acid, resulted in inactivation of the clotting mechanism. The test tubes (10 x 100 mm) used were left in cleaning solution for 24 hours, rinsed 3 times with tap water followed by three rinsings with distilled water, then dried and stored until used. Coagulation studies were performed as given in Table I, the solutions mixed in the order of their column. Prior to mixing, the separate solutions were kept in an ice bath at about 1°C and after mixing in a water bath at 37.5°C for 60 seconds. All determinations were performed in triplicate and the end point used was the first appearance of fibrin threads. These threads are easily seen when direct light passes through the solution.

Results. The protocol of a typical experiment showing the effect of Toluidine Blue upon normal and heparinized rabbit plasma is given in Tables II and III. Graph 1 illustrates the effect of the seven dyes investigated upon the clotting times of normal and heparinized rabbit plasma. In all cases the dyes tended to produce a rapid inactivation of heparin although the amount of dye required to return the clotting time to its normal level varied with the particular dye used. It is apparent that the amount of dye required for heparin inactivation is critical because small

3. MacIntosh, F. C., *Biochem. J.*, 1941, v35, 776.
4. Grunke, W., *Klin. Woschschr.*, 1939, v18, 443.
5. Grunke, W., *Z. ges. exp. Med.*, 1940, v107, 306.
6. Herrmann, H., *Skand. Arch. Physiol.*, 1937, v76, 125.
7. Astrup, T., *Enzymologia*, 1938, v5, 12.
8. von Jeney, A., Valyi-Nagy, T., and Magyari, J., *Arch. exp. Path. Pharmakol.*, 1940, v196, 505.
9. Lewis, M. N., and De Maria, F., *J. Am. Pharm. Assn., Sci. ed.*, 1949, v38, 441.
10. Astrup, P., *Acta Pharmacol.*, 1947, v3, 165.

TABLE I.
Order of Mixing of Coagulation Systems.

Tube	Plasma, cc	Thrombo-plastin, cc	Distilled water, cc	Heparin, cc	Dye, cc	CaCl, cc
Control	.1	.05	.1	0	0	.2
Heparin	.1	.05	0	.1	0	.2
Dye	.1	.05	0	0	.1	.2

TABLE II.
Coagulation Time in Seconds of Plasma and Toluidine Blue.

Concentration of dye, μg/0.1 cc (0.85% NaCl)	0	5	10	15	25	50	75	100	125	150	200
1st series of tubes	92	89	92	92	104	135	191	215	298	330	376
2nd series of tubes	92	86	90	93	105	136	186	213	293	319	376
3rd series of tubes	93	87	94	97	107	138	195	209	289	327	382
Avg time in sec.	92	87	92	94	105	136	191	212	293	325	378

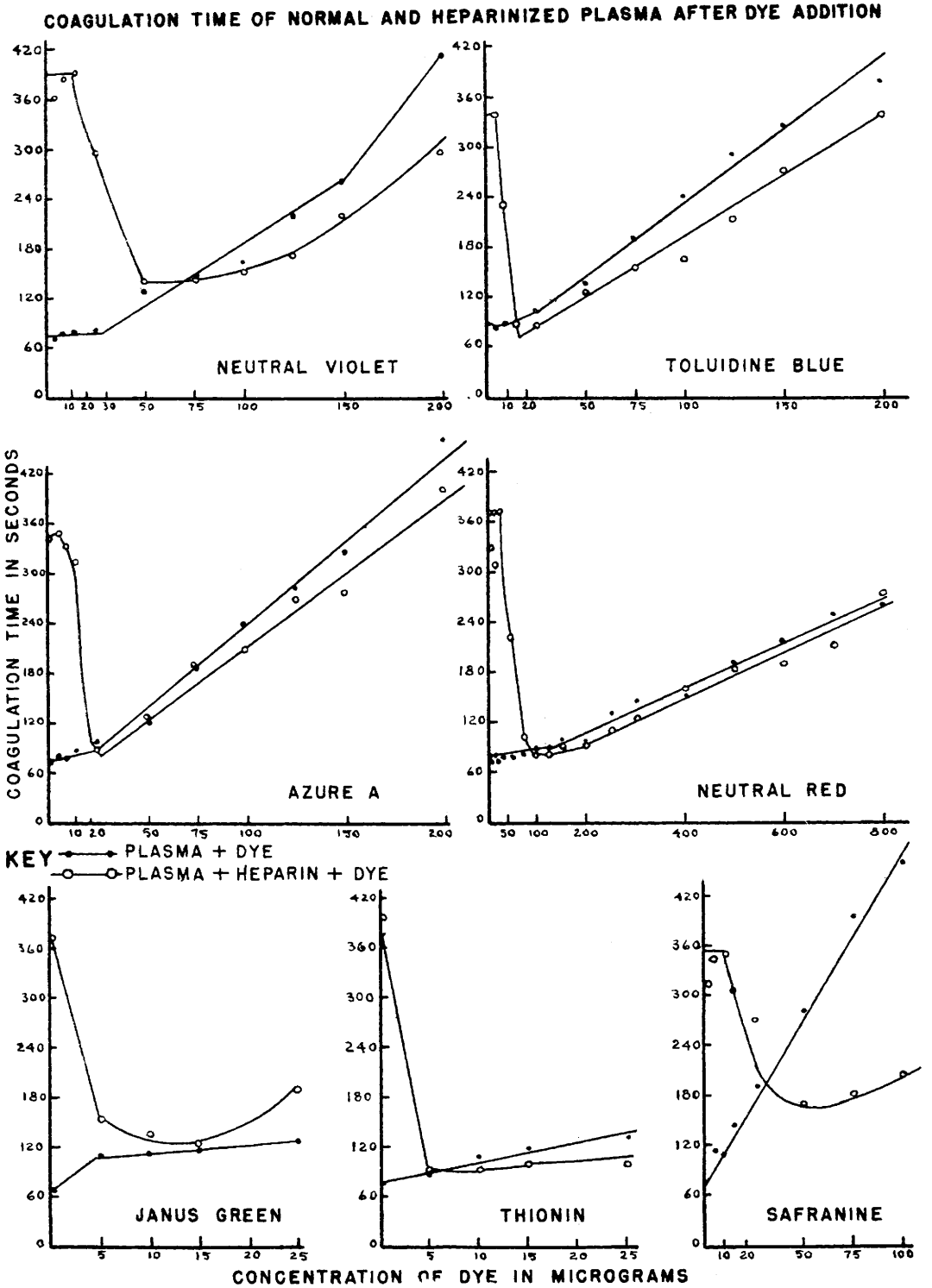
TABLE III.
Coagulation Time in Seconds of Plasma, Heparin, and Toluidine Blue.

Concentration of dye, μg/0.1 cc (0.85% NaCl)	0	5	10	15	25	50	75	100	125	150	200
1st series of tubes	350	353	293	89	96	136	170	172	205	270	293
2nd series of tubes	354	356	296	88	100	140	165	169	217	268	291
3rd series of tubes	355	350	290	91	98	142	164	173	217	272	308
Avg time in sec.	353	353	293	89	98	139	166	171	212	270	297

amounts do not greatly reduce clotting time, whereas larger quantities prolong clotting time. Inspection of the curves in Graph 1 shows that after dye addition beyond the critical point, except in the cases of Janus Green, Thionine and Safranine, the curves for both the normal and heparinized plasmas are essentially parallel. This would indicate that these dyes have an anticoagulant effect and that the excess dye is acting the same in both cases and upon some substance in the system other than heparin.

Discussion. It has been reported previously that when small amounts of Toluidine Blue (4,5) are added to heparinized blood the coagulation time is prolonged. We were unable to confirm these findings; in fact, we found that this dye had no effect on normal plasma at concentrations of 5 and 10 μg. However, this latter concentration markedly reduced the coagulation time of heparinized plasma. The anticoagulant effect observed with increasing concentrations of Toluidine Blue(4,5) and Janus Green(6-8) is essentially the same as reported herein. Herrmann(6) reported that Neutral Red did not increase coagulation time but we found that this dye behaved as an anticoagulant when the concentration was sufficiently high. The results herein reported for Neutral Red and Safranine confirm those of von Jeney, *et al.*(8) However, those investigators worked with only one concentration of dye, 100 μg per 0.1 cc, and therefore did not observe the anticoagulant effects we are reporting. It appears from the results herein presented that the indiscriminate use of these dyes in heparinemia would be highly detrimental to the welfare of a patient. However, the *in vivo* effects of these dyes may not be entirely the same as the *in vitro* effects.

Summary. A study has been made of the effect of 7 dyes of the phenazine, oxazine and thiazine series on the *in vitro* coagulation times of normal and heparinized rabbit plasma. Toluidine Blue, Azure A, Neutral Violet and Neutral Red effectively counteract the anticoagulant action of heparin but the amount of each dye required for this antiheparin effect is


COAGULATION TIME OF NORMAL AND HEPARINIZED PLASMA AFTER DYE ADDITION
COAGULATION TIME IN SECONDS
NEUTRAL VIOLET
TOLUIDINE BLUE
AZURE A
NEUTRAL RED
JANUS GREEN
THIONIN
SAFRANINE
KEY PLASMA + DYE
PLASMA + HEPARIN + DYE
CONCENTRATION OF DYE IN MICROGRAMS

GRAPH 1.

critical. At concentrations below the critical level the dyes had little or no effect upon the coagulation system studied. At concentrations above the critical level all the dyes produced a progressively increased incoagulability of the system. This anticoagulant effect of the dyes is related to concentration and the action is on some substance other than heparin.

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Toxicity of Some Synthetic Pteridines in Rats.* (17593)

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This is an extension, into the field of vitamin analogs, of the research started in this laboratory(1) on the effect of amino acid analogs on tumor-bearing rats and mice. While there is considerable literature on the anti-folic acid activity and tumor-inhibiting effects of such folic acid analogs as 4-amino-pteroylglutamic acid and 4-amino-N-10-methyl pteroylglutamic acid, less work has been done with the substituted pteridines themselves. The importance of the 4-amino substitution for folic-acid antagonism has been demonstrated in much of this work.(2) In this connection, the anti-folic acid activity and tumor-inhibiting effect of 2,6-diamino-purine is of interest.(3) This compound is identical with 2, 4-diamino-pteridine except for the extra carbon atom in the pteridine ring. In view of these facts, several 4-amino substituted pteridines were prepared in this laboratory.(4) A study of the toxicity of these compounds in normal rats was undertaken before investigating their effects on tumor-bearing animals.

Procedure. For this study, 56 female rats of the Long-Evans strain weighing about 150 g were used. They were divided into 7 groups of 8 animals each. Two groups served as controls, while each other group received one of the 5 different pteridines. They were kept on the regular stock diet during the experimental period. The compounds were dissolved in 0.1 M phosphate buffer, pH 7.4, and the concentrations used, except for 2,4-diamino-pteridine, approached the maximum solubility.

Of the eight animals used for each compound studied, 4 received 3 ml intraperitoneally daily (Low Dose), and 4 received 3 ml twice daily (High Dose). One control group was not injected; the other was given the same volume of phosphate buffer as the experimental animals. The injection period was for 10 days. Half of each group was killed on the eleventh day (Group A), and the rest were killed 6 days later (Group B). At the time of sacrifice, blood was taken for hemoglobin, white blood count and blood smear. The kidneys and liver were weighed, and pieces of kidney, liver and sternal marrow were fixed in formalin. A bone marrow smear was made by

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1. Greenberg, D. M., and Schulman, M. P., *Science*, 1947, v106, 271.

2. Daniel, L. J., Norris, L. C., Scott, M. L., and Heuser, G. F., *J. Biol. Chem.*, 1947, v169, 689.

3. Hitchings, G. H., Elion, G. B., Vander Werff, H., and Falco, E. A., *J. Biol. Chem.*, 1948, v174, 765; Burchenal, J. H., Bendich, A., Brown, G. B., Elion, G. B., Hitchings, G. H., Rhoads, C. P., and Stock, C. C., *Cancer*, 1949, v2, 119.

4. Gal, E. M., *J. Am. Chem. Soc.*, (in press).