than intra-arterial injection, as would be anticipated. On the other hand, standardized intravenous injection of epinephrine (1-20 μg) and mecholyl (.1-1.0 μg) produced little or no detectable flow response through the adrenal gland (mecholyl demonstrated a barely discernible flow increase at higher dosages of 10 μg). This readily apparent difference in vascular response from ACTH, epinephrine, and mecholyl in the adrenal vasculature as compared to the periphery deserves a more thorough study.

The histological finding that a "normal" gland, as revealed by microscopic examination of a hematoxylin and eosin preparation, does not secrete 17-hydroxycorticosteroids and is refractory to ACTH raises doubts as to the validity of many conclusions based on only microscopic appearances. It indicates that action of DDD has a latent period and emphasizes the uncertainty of judging the secretory status of the adrenal from microscopic examination. The finding that after 18 days a normal secretory pattern and response to ACTH is present in dog adrenals with cytological change is particularly interesting since Vilar and Tullner(7) have shown that after 4 days treatment with the o,p'-isomer histological changes are apparent, and secretion of corticosteroids is depressed, indicating that secretory capacity recovers while mild histological alteration continues. This delayed histological change from 3 doses of technical DDD has not heretofore been reported.

Conclusions. Technical DDD fed to dogs in 200 mg/kg doses once daily for 3 days resulted in histologically normal adrenal glands which responded to ACTH with the expected increase in blood flow, but demonstrated a low resting secretion rate of 17-hydroxycorticosteroids, which did not increase after administration of ACTH. Dogs fed a similar 3 doses and allowed to continue normal diet for 18 days showed adrenals with definite changes in the cortex which did respond to ACTH with the expected increase of steroid secretion but showed usual increase in blood flow. These data are interpreted to mean that the vasodilating factor of commercial ACTH is separate from steroid formation and/or releasing factor(s) and possibly the vasodilating factor may act on medullary or extracapsular vasculature. Capacity of the cortical parenchymal cells to form and/or release 17-hydroxycorticosteroids, as influenced by DDD, does not parallel histological changes observed in standard hematoxylin and eosin preparations.

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Interaction of Chlorpromazine with Strandin.* (25902)

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LeBlanc(1,2) has shown that chlorpromazine can destroy the mast cell, and has suggested that some of the effects of this drug may be due to release of histamine and serotonin from that cell. The mast cell is also considered to be the site of heparin production(3). During recent study concerned with metachromasy of brain ganglioside, strandin

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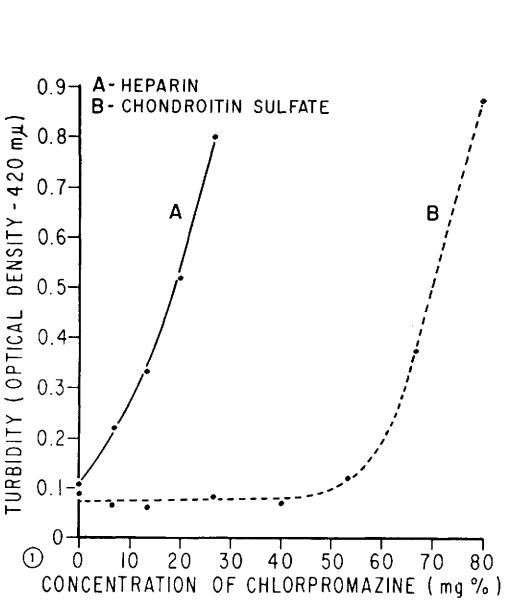
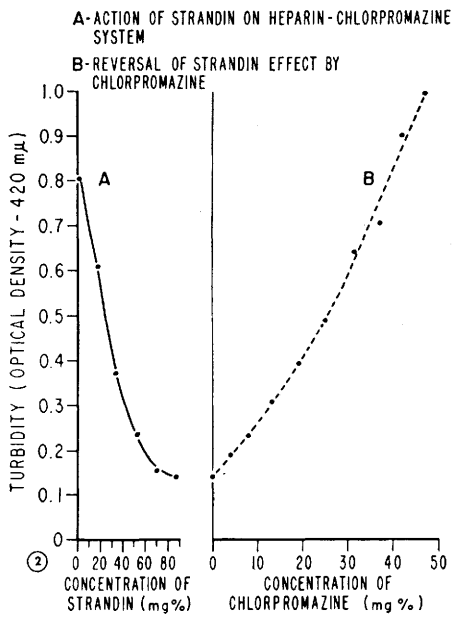
(4), the similarity in structure between thiazine dyes and phenothiazine drugs was noted. Aggregation and micelle formation among metachromatic dyes is a well known phenomenon(5). Scholtan(6) studied aggregation among phenothiazine derivatives which contain alkylated ammonium groups, and concluded that these compounds behave as colloidal electrolytes in solution. Our preliminary experiments indicated that heparin and chrondroitin sulfate interact with some phenothiazine tranquilizers to form products of visible turbidity. In addition, strandin and the sulfated mucopolysaccharides (heparin and chondroitin sulfate) share certain common biochemical properties, such as inhibition of clotting mechanism(7,8), metachromasy(4,8) and interaction with basic proteins (9). It was considered of interest to determine whether a reaction occurs between phenothiazine tranquilizers and strandin.

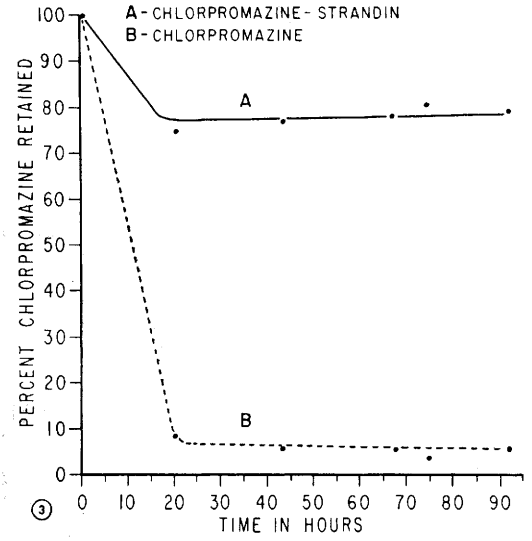
Materials and methods. 1. The brain ganglioside, strandin, was extracted from the cortical gray matter of a case of Tay Sachs' disease, by the method of Folch, Lees and Sloane-Stanley(10). Tissue was maintained at −8°F in the deep freeze, prior to extraction. Strandin was analysed by the neuraminic acid method of Saifer and Gerstenfeld(11), assuming an average value of 30.5 mg of neuraminic acid per 100 mg of strandin(12). 2. Chlorpromazine and trifluoperazine (Stelazine) were obtained from Smith, Kline and French Laboratories, Philadelphia, Pa.† 3. Heparin (Sodium salt, 100 International Units per mg) was obtained from Connaught Medical Research Labs., Univ. of Toronto, Canada. 4. Chondroitin sulfate (CSA) was obtained from Pentex Inc., Kankakee, Ill. 5. Lysozyme (2× crystallized) was obtained from Worthington Biochemicals Corp., Freehold, N. J. 6. Toluidine Blue O (Total dye content of 94%) was obtained from Nat. Aniline Division, Allied Chemical and Dye Corp., N. Y. 7. All other chemicals used were of reagent grade. 8. All measurements of turbidity were made using the Bausch and Lomb Spectronic-20 spectrophotometer, set at a wavelength of 420 mμ. 9. Measurements of metachromasy were made with the same instrument using as an index of metachromasy the Optical Density Ratio (O.D.R.), which is equal to (O.D. 550/O.D. 635)(4). 10. Measurements of ultraviolet absorption spectra were made with Beckman DU Spectrophotometer. 11. Ultracentrifuge patterns were obtained with Spinco Ultracentrifuge-Model E, maintained at a velocity of 50,740 rpm, at 20°C. 12. Dialysis equilibrium was performed with "Visking" cellulose casings. Chlorpromazine remaining within the sac during period of dialysis was analysed by a colorimetric procedure described below.

Results. A) *Turbidimetric studies.* Phenothiazine derivatives form products of visible turbidity with heparin and chondroitin sulfate (CSA), quantitatively demonstrated in Fig. 1. Increased optical density values at 420 mμ, indicating increased turbidity, were used as the index of this interaction. Heparin reacts more readily than CSA, the latter requiring addition of a higher concentration of chlorpromazine before turbidity becomes optically apparent. Absorption at zero chlorpromazine concentration is due to the slight color of concentrated mucopolysaccharide solutions and not to turbidity. Qualitatively similar results were obtained with stelazine. When strandin (0.87%) is added to the heparin-chlorpromazine reaction product, there is a progressive decrease in turbidity, with increasing concentration of strandin, so that on addition of 2.61 mg (88 mg%) of strandin turbidity is reduced from 0.810 to 0.145, an 82.1% decrease (Fig. 2A). Upon titration of this system with chlorpromazine, turbidity reappears (Fig. 2B). Similar results were obtained on treating chondroitin sulfate-chlorpromazine with strandin, turbidity reappearing following titration with chlorpromazine. Results of both experiments were duplicated with stelazine. It has been demonstrated that strandin interacts with basic proteins(9); and metachromasy of strandin was found to be extremely sensitive to changes in the physicochemical environment(4). Turbidity of the

† We thank M. J. Ritchie, Med. Dept., Smith, Kline and French Labs., for providing a generous supply of these compounds.







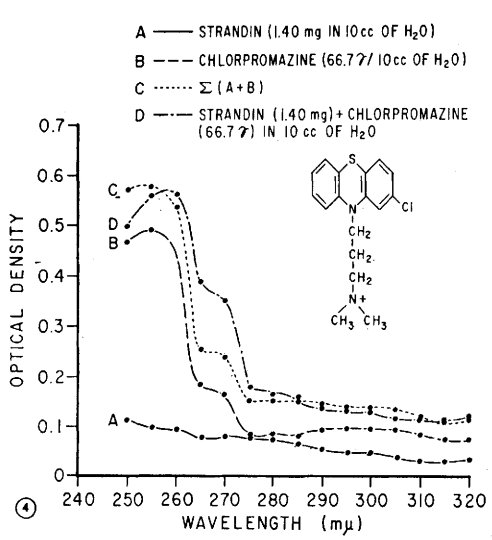


FIG. 1. Interaction of chlorpromazine with heparin (A) and chondroitin sulfate (B). 2 ml of a 2% solution of acid mucopolysaccharide was reacted with amount of chlorpromazine indicated, in water. Vol constant at 3 ml. Temp. 26°C. pH change for heparin system 6.18 to 6.15 and for CSA 6.10 to 5.80.

FIG. 2. Reversible repression of heparin-chlorpromazine turbidity. A: 2 ml of 2% heparin plus 0.2 ml of 0.4% chlorpromazine plus indicated quantity of strandin (0.87%) in water. Vol constant at 3 ml. Temp. 26°C. pH change from 6.50 to 6.20. B: Titration of 2 ml of 2% heparin plus 0.2 ml of 0.4% chlorpromazine plus 0.3 ml of 0.87% strandin with chlorpromazine (0.4%) in water. Final vol 3.4 ml. Temp. 26°C. No pH change. (Final pH 6.20.)

FIG. 3. Equilibrium dialysis of chlorpromazine and chlorpromazine-strandin at 4.4°C. A: Chlorpromazine-strandin: 2 ml portions of 0.1% chlorpromazine plus 0.87% strandin, each dialysed against 30 ml of water. pH change within dialysis sacs was from 6.35 to 6.12. B: Chlorpromazine: 2 ml portions of 0.1% chlorpromazine; each dialysed against 30 ml water. pH change within dialysis sacs was from 5.42 to 6.51.

FIG. 4. Effect of strandin on ultraviolet absorption spectrum of chlorpromazine. A: 0.4 ml of 0.35% strandin (1.40 mg) in 10 ml of water. B: 10 ml of chlorpromazine (66.7 γ). C: Algebraic sum of absorption of A and B, Σ (A + B). D: 0.4 ml of 0.35% strandin (1.40 mg) plus 66.7 γ of chlorpromazine in 10 ml of water.

lysozyme-strandin interaction product is reduced by chlorpromazine. After titration of this chlorpromazine-treated system with strandin (0.4%) turbidity is partially regenerated. Metachromasy of the toluidine blue O-strandin system, as measured by the O.D.R. (O.D. 550/O.D. 635) is reduced by about 71.0% after titration with chlorpromazine.

B) *Equilibrium dialysis.* To obtain further proof of the proposed interaction between chlorpromazine and strandin, 5 (2 ml) solutions of chlorpromazine and chlorpromazine plus strandin were placed in dialysis sacs ("Visking" cellulose casing) in 30 ml of water. Initial concentration of chlorpromazine was 0.1% (2 mg/2 ml) and 0.87% strandin (17.4 mg/2 ml). Internal samples were analysed at intervals by the colorimetric method of Leach and Crimmin(13), employing ferric nitrate as the oxidizing agent and reading the resulting red solution at 530 mμ with the spectrophotometer. Results were computed from a standard curve as mg/l of chlorpromazine inside the dialysis sac, then converted to percent chlorpromazine retained by the sac. The series of experiments conducted without strandin shows a rapid loss of chlorpromazine from the internal phase of the system (Fig. 3B), so that only about 7.5% remains after the first 20 hours. At end of 92 hours, the internal phase has retained but 5.5% of original amount of chlorpromazine. In the system which contains strandin (3A), marked retention of chlorpromazine occurs. Thus in the first 20 hours, this system has retained 77.5% of the original amount of chlorpromazine, and this level of internal concentration is maintained throughout the remaining 72 hours of experiment, as indicated by analysis of the remaining 4 sacs.

C) *Effect of strandin on ultraviolet absorption spectrum of chlorpromazine.* Fig. 4A shows ultraviolet absorption of 0.4 ml of 0.35% strandin (1.40 mg) in 10 ml of water. At this concentration little absorption is demonstrable. Fig. 4B represents absorption spectrum of chlorpromazine (66.7 γ/10 ml). Fig. 4C demonstrates the algebraic sum of A (strandin) plus B (chlorpromazine) absorption. It is the theoretical absorption which should result from a mixture of the 2 substances in absence of interaction. The observed curve resulting from mixture of 0.4 cc of 0.35% strandin (1.40 mg) in 10 ml of water containing 66.7 γ of chlorpromazine is indicated in Fig. 4D. When compared with the theoretical curve (Fig. 4C), maximum optical density has shifted from 255 mμ to 258 mμ. In addition overall absorption in the range 257.5 mμ to 285 mμ is greater than that of the theoretical curve. Beyond 275 mμ the changes are small and do not appear to be of great significance. The shift in absorption maximum of stelazine is greater, shifting from 256 mμ to 266 mμ for the observed system (0.4 ml of 0.35% strandin (1.40 mg) in 10 ml of stelazine (100 γ/10 ml). There is a noticeable increase in absorption in the range of 262.5 mμ to 272.5 mμ, in the case of the observed as opposed to the theoretical curves. Beyond 272.5 mμ changes are small.

D) *Effect of chlorpromazine on ultracentrifuge pattern of strandin.* The following solutions were analysed in the ultracentrifuge (50,740 rpm and 20°C) using an analytical cell with a one degree prismatic window. (a) 0.44% strandin in water adjusted to pH 5.0 with 0.1N HCl, (b) 0.44% strandin in 0.5% chlorpromazine at pH 5.0. The results of this experiment are indicated in Fig. 5A and 5B. Upper portion of the diagrams represents the pattern of strandin alone, lower portion indicates the pattern resulting from action of chlorpromazine. Fig. 5A represents the pattern at commencement of experiment, and 5B the pattern after 64 minutes.

At beginning of the run, the strandin pattern shows 2 peaks (a phenomenon not found at pH 6.7); after 64 minutes only one peak of smaller size is noted. A single large peak is found in the chlorpromazine-strandin mixture at beginning of the run; after 64 minutes a peak of medium size is found preceded by a shoulder which appears to be coincident with that found in the upper pattern representing strandin alone. With a solution of 0.5% chlorpromazine in water, (pH 6.0), no peaks were observed. It seems probable that the medium-sized peak, shown by the strandin-chlorpromazine mixture (5B), represents a

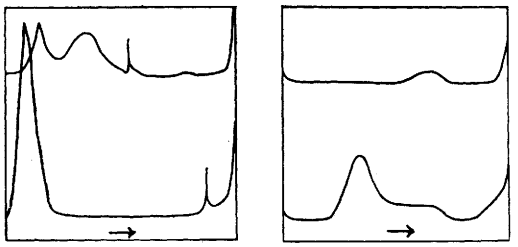


FIG. 5. Ultracentrifuge patterns of strandin (upper) and strandin plus chlorpromazine (lower), taken at commencement of run (5A) and after 64 min. (5B). Samples were spun at 50,740 rpm at 20°C. Strandin solution consists of 0.44% of the ganglioside in water at pH 5. Mixture consists of 0.44% strandin in 0.5% chlorpromazine at pH 5. The arrow above the abscissa indicates the direction of movement of sedimenting boundary.

slow moving complex of strandin with chlorpromazine.

Discussion. Chlorpromazine functions as an inhibitor of a number of enzyme systems. Thus it suppresses oxidative phosphorylation (14), cytochrome oxidase(14) ATPase(14) and D-amino acid oxidase(15). Yagi has presented evidence indicating that complex formation occurs between chlorpromazine and flavine adenine dinucleotide(16). LeBlanc (1,2) has indicated that chlorpromazine can destroy the mast cell. The present studies suggest that chlorpromazine and stelazine can interact with heparin and CSA to form products of visible turbidity.

Little is known concerning the physiological function of strandin, aside from its inhibition of the blood clotting mechanism(8) and influenza virus hemagglutination(17). It reacts with basic dyes(4) and basic proteins (9), most probably *via* the carboxyl groups of the neuraminic acid moiety of the molecule. It seems likely that this grouping is responsible for its interaction with chlorpromazine, the latter reacting *via* its positively charged nitrogen. This, however, is speculative, and further evidence will be required to ascertain the nature of the bond existing between these substances. Interaction of strandin with chlorpromazine and stelazine may have a bearing on mode of pharmacological activity of the phenothiazine derivatives. It is thought that these drugs primarily act on the midbrain (18). Since concentration of strandin is greater in the gray matter of the cortex, the presence of strandin may serve to protect the cells of the cortex from the drug.

Summary. 1) Chlorpromazine and stelazine interact with heparin and CSA to form products of visible turbidity. This turbidity is reduced by strandin, and reappears on readdition of phenothiazine. 2) Chlorpromazine reduces turbidity of the strandin-lysozyme interaction product, turbidity of which may be regenerated by subsequent addition of strandin. It also destroys metachromasy of the toluidine blue O-strandin system. 3) Equilibrium dialysis of chlorpromazine solutions and chlorpromazine-strandin solutions indicates good retention of chlorpromazine in the system containing strandin. 4) Strandin alters U.V. absorption spectrum of chlorpromazine and, more markedly, that of stelazine. 5) Ultracentrifuge studies indicate presence of slow moving peak in the strandin-chlorpromazine system, which may represent strandin-chlorpromazine interaction product.

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Microbial Resistance to Penicillin as Related to Penicillinase or Penicillin Acylase Activity. (25903)

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Presence of enzyme penicillinase that specifically destroys penicillin activity by degradation to inactive penicilloic acid, offers a ready explanation for resistance of certain microorganisms to this antibiotic. With recent discovery(1) that many microorganisms are able to cleave the acyl group in penicillin G, to yield the relatively inactive moiety 6-aminopenicillanic acid (6-APA), still another mechanism for penicillin resistance became apparent. A number of bacteria are reported here to relate their resistance to penicillin to either penicillinase or penicillin acylase activity.

Results. It is obvious that 6-APA possesses definite antibacterial properties, but to a much less degree than penicillin G (Table I). Comparative values expressed as minimum inhibitory concentration (MIC) in μg/ml in Table I indicate that 6-APA is 4166-fold to 390-fold less active than penicillin G when tested *in vitro* against a series of Gram positive bacterial genera. Although the differences against the Gram negative bacteria are not so striking, 6-APA is 2-fold to 10-fold less active than penicillin G. Differences in activity similar to this were obtained *in vivo*. At concentrations of 200 mg/kg 6-APA failed to protect mice against experimental *Staphylococcus aureus* infections, and levels of 50 mg/kg against *Streptococcus pyogenes* infections offered no protection. In contrast, penicillins G and V were highly effective (Table I).

The relationship of MIC to penicillin, and presence or absence of penicillinase or penicillin acylase activity, for a number of different bacteria are shown in Table II. After an average MIC for penicillin was established, bacterial cells of each culture were examined for presence of penicillin ăcylase activity, *i.e.,* hydrolytic conversion of penicillin G to 6-APA, by the chromatographic procedure described by Batchelor(2). In addition, supernates were examined by several methods for evidence of biological inactivation of penicillin. In one system sterile filtrates of 24 hour cultures were achieved by passage through sin-

TABLE I. Comparison of Activity of 6-Aminopenicillanic Acid and Penicillin G.

I. *In Vitro*	MIC, μg/ml*	
Microorganism	6-Aminopenicillanic acid	Penicillin G
Staphylococcus aureus	125	.03
Streptococcus pyogenes	15.6	.006
" *faecalis*	250	.16
Diplococcus pneumoniae	250	.0078
Erysipelothrix rhusiopathiae	125	.08
Corynebacterium diphtheriae	31.2	.09
Bacillus subtilis	62.5	.02
" *cereus*	125	.04
Aerobacter aerogenes	62.5	25
Escherichia coli	6.25	25
Proteus vulgaris	250	25
Pseudomonas aeruginosa	250	100
Salmonella typhosa	125	12.5
" *pullorum*	62.5	12.5
Klebsiella pneumoniae	62.5	12.5
Neisseria gonorrheae	1.9	.19
Shigella sonnei	125	50
Brucella bronchiseptica	125	100

II. *In Vivo*	PD_{50}, mg/kg			
	6-Aminopenicillanic acid		Penicillin†	
Infection in mice‡	Oral	I.V.	Oral	I.V.
Staphylococcus aureus	>200	>200	1.5	.78
Streptococcus pyogenes	>50	>50	.9	.6

* 2-fold 10 tube serial dilution technic—MIC read after 20 hr incubation at 37°C.

† Penicillin V used orally; penicillin G used intravenously.

‡ Intraper. infection—treatment started ½ hr later—single dose. PD_{50} calculated after 96 hr.