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Refined Hyperimmune Rickettsial Sera.

TIMOTHY J. KUROTCHKIN, J. VAN DER SCHEER AND RALPH W. G. WYCKOFF.

From Lederle Laboratories, Pearl River, N. Y.

Topping[1] has demonstrated that monkeys and guinea pigs fatally infected with Rocky Mountain spotted fever can be caused to recover by the administration of hyperimmune rabbit serum. This naturally suggests that such serum may have value in the treatment of human cases. The sera prepared by Topping were made by the injection of infectious tick-tissue as antigen. We have found that a serum of high neutralizing titer can also be obtained by injecting rabbits with yolk-sac material prepared according to the method of Cox.[2] We have determined that this can be refined by the same chemical procedures which are useful in purifying antipneumococcal rabbit serum and removing its toxic and reaction-producing elements. In such concentrates the neutralizing titer per gram of protein is increased about 20-fold as compared with the original serum. A similar antiserum and concentrate of high neutralizing value has also been made against the Breinl strain of European typhus. Sufficient quantities of these two sera are now being prepared and refined so that a thorough clinical study of their possible effectiveness in the treatment of Rocky Mountain spotted fever and epidemic typhus fever in man can be made.

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Fate of the Lethal Effect of Sublethal Doses of Lanatoside C, Digoxin and Digoxigenin.

ARTHUR C. DEGRAFF AND ROBERT A. LEHMAN.

From the Department of Therapeutics, New York University, College of Medicine.

The availability today of many of the "genuine" and intermediate glycosides and of the genins of the digitalis group has made possible the study of the relationship between structure and pharmacologic action. The importance of this study lies in the possible therapeutic

1 Topping, N. H., *U. S. Pub. Health Rep.*, 1940, **55**, 41.

2 Cox, H. R., *U. S. Pub. Health Rep.*, 1938, **53**, 2241.

use of the pure substances, thus avoiding the approximations of bioassay, and also in the insight which may be given to the action of the galenicals. With this in mind a comparison has been made of the fate of the lethal effect of sublethal doses of lanatoside C, digoxin and digoxigenin in the cat. This series was chosen because of the chemical relationship of the 3 drugs. Thus, lanatoside C is the "genuine" glycoside as it occurs in digitalis lanata, digoxin the product of partial hydrolytic cleavage of lanatoside C, and digoxigenin is the genin common to both. The chemical relationships are reviewed by Stoll.[1]

The method was essentially that originally described by Hatcher[2] and since used by many other workers (Rothlin,[3, 4] van Esveld,[5] Bauer,[6] Gold,[7] Chen,[8] Hoekstra[9]). It consists in injecting intravenously an accurately measured sublethal dose of the glycoside to be studied and, after varying time intervals, infusing ouabain until the death of the animal. It will be noted that this procedure has been applied by various investigators in the study of "cumulation," and "rate of excretion" of the digitaloids. These phrases are avoided since their definition depends upon the arbitrary assumption of a mechanism of action.

As a preliminary step the 3 glycosides were assayed by the usual Hatcher and Brody[10] technic, using a concentration of 1:100,000 for digoxin and lanatoside C, 1:25,000 for digoxigenin, and 1:200,000 for ouabain, and a rate of injection such that all animals died between 30 and 90 minutes after the start of the infusion. Using 10 to 20 cats for each substance, the geometric mean lethal dose of lanatoside C was found to be 0.261, digoxin 0.335 and digoxigenin 0.538 mg per kg. These results do not confirm Rothlin's[11] surprising finding that digoxigenin is more toxic than digoxin, the glycoside from which it is obtained, but are in agreement with Smith[12] and Chen[8] who found digoxin to be the more potent. It

1 Stoll, A., *J. Am. Pharm. A.*, 1938, **27**, 761.
2 Hatcher, R. A., *Arch. Int. Med.*, 1912, **10**, 268.
3 Rothlin, E., *Munch. med. Wchschr.*, 1933, **80**, 726.
4 Rothlin, E., *Annale de l'Hospital de la Santa Creu i Sant Pau*, 1935, **9**, 586.
5 Esveld, L. W., *Arch. f. exp. Path. u. Pharmakol.*, 1931, **160**, 375.
6 Bauer, H., *ibid.*, 1934, **176**, 65.
7 Gold, H., *Arch. Int. Med.*, 1923, **32**, 779.
8 Chen, K. K., Robbins, E. B., and Worth, H., *J. Am. Pharm. A.*, 1938, **27**, 189.
9 Hoekstra, R. A., *Arch. f. exp. Path. u. Pharmakol.*, 1932, **163**, 35.
10 Hatcher, R. A., and Brody, J. G., *Am. J. Pharm.*, 1910, **82**, 360.
11 Rothlin, E., *Schweiz. med. Wchschr.*, 1938, **68**, 1336.
12 Smith, S., *J. Chem. Soc.*, 1930, **133**, 508.

will be noted from the table that calculation of the assays in terms of micro-moles per kg accentuates this difference in activity.

Initial doses of 0.20 mg per kg for lanatoside C and digoxin and 0.34 mg per kg for digoxigenin respectively were then injected into the femoral vein. The magnitude of the preliminary injection was made as large as possible so that the effect measured would be significantly greater than the variations about the mean, but not so large as to kill any of the animals within 24 hours. The arbitrary adjustment used by Gold[7] for difference in susceptibility was thus avoided.

The variations in the lethal effect were determined for 3 or 6 and 24 hour intervals. Eight to 10 cats were sacrificed for each time interval. The concentration of the ouabain solution for the final titration was 1:200,000 in saline and the rate of injection was one to 2 cc per minute. No general anesthetic was used at any time. Rothlin,[4] Bauer,[13] Heubner and von Nyary[14] have objected to the Hatcher procedure on the ground that after a large but sublethal dose of a digitaloid, cats lose considerable weight and develop pathological changes in the myocardium. They maintain that when ouabain is infused 2 to 14 days after an initial injection, the amount required is not a function of the time interval, nor of the magnitude of the preliminary dose, but rather of the extent of the pathologic changes in the heart and the state of nutrition of the animal. For time intervals of less than 48 hours, however, no pathology of the heart was observed and the state of nutrition of the animal would not be expected to influence the experiments. It is probable, therefore, that the method of Hatcher is capable of following early changes in the lethality of these drugs in the animal body.

The results may be calculated a number of ways without altering the conclusions. The following expression,

$$\frac{100[(\text{Initial Dose}) + (\text{Final Dose}) - 1]}{(\text{Initial Dose})}$$

gives the percentage of the lethal effect of the initial dose which is dissipated after the stated interval, where the dosages are expressed as fractions of the Hatcher and Brody lethal dose (Table). The animals varied in weight from 2.5 to 4.0 kg. The mean weight of the entire 112 cats was 3.1 kg. The results are shown graphically in the figure.

Conclusions. The lethal effect of lanatoside C dissipates rapidly

[13] Bauer, H., and Fromherz, K., *Klin. Wchschr.*, 1933, **12**, 973.

[14] Heubner, W., and v. Nyary, A., *Arch. f. exp. Path. u. Pharmakol.*, 1934, **177**, 60.

TABLE I.
Geometric Mean Lethal Dose of Lanatoside C, Digoxin and Digoxigenin by the Hatcher and Brody Assay.

Glycoside	Empiric formula*	Molecular wt	Lethal dose: Mg per kg	Lethal dose: Micro mols per kg	Values in the literature mg per kg†
Lanatoside C	$C_{49}H_{76}O_{20}$	984	0.261	0.265	0.255 Rothlin[11] 0.280 Rothlin[11]
Digoxin	$C_{41}H_{64}O_{14}$	780	0.335	0.430	0.285 Rothlin[4] 0.22 Chen[8] 0.442 White[15]
Digoxigenin	$C_{23}H_{34}O_5$	390	0.538	1.38	0.253 Rothlin[11] 0.473 Chen[8]
Ouabain	$C_{29}H_{44}O_{12}$	584	0.102	0.171	0.10 to 0.12 numerous observers

*Stoll.[1]

†All of these values are for the cat by intravenous infusion but some were obtained by modifications of the Hatcher and Brody technic.

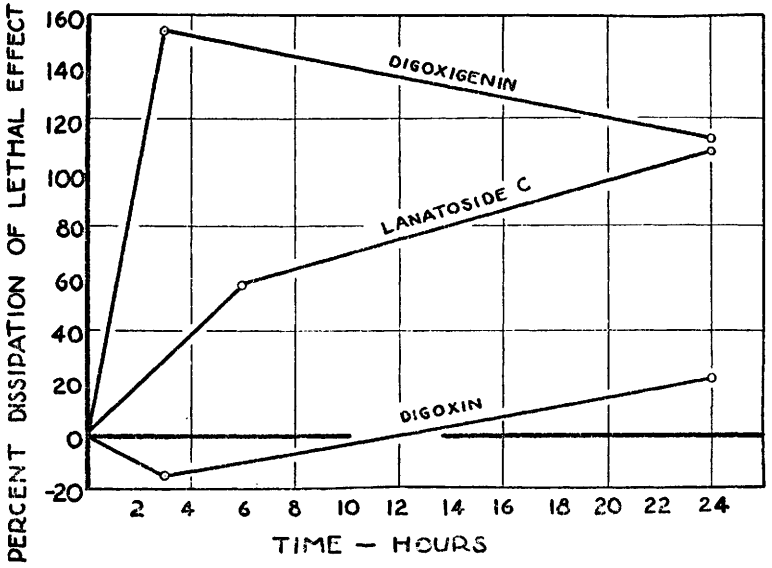

FIG. 1.
Per cent dissipation of the lethal effect of sublethal doses of lanatoside C, digoxin and digoxigenin with time.

during the first 24 hours after intravenous injection in the cat. The lethality of digoxin, however, actually increases during the first three hours and is still quite strong after 24 hours. This very considerable qualitative difference between the action of the "genuine" glycoside and the product of its partial hydrolytic degradation

[15] White, A. C., *J. Pharmacol.*, 1934, **52**, 1.

appears not to have been emphasized before. As would be expected, the effect of digoxigenin completely disappears in 24 hours. At 3 hours, however, digoxigenin causes an increased tolerance for ouabain. Further investigation is needed to determine the significance of this finding.

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Cultivation of Virus of Equine Encephalomyelitis in Serum-Ultrafiltrate and Buffered Salt Solution (Simms).*

MURRAY SANDERS AND ELEANORA MOLLOY.†

From the Departments of Bacteriology and Dermatology, College of Physicians and Surgeons, Columbia University, New York City.

Propagation of a virus in protein-free serum-ultrafiltrate cultures was first accomplished with the agent of lymphogranuloma venereum.[1] The same technic has since been successfully applied to St. Louis encephalitis[2] and a murine strain of poliomyelitis.[3] In the previous studies emphasis was placed upon the stability of the serum-ultrafiltrate cultures as manifested by consistent pH's ranging approximately between the neutral point and 7.6, by persistent viability of tissue cells for long periods of time and proportionate maintenance of high viral potencies. In 2 of the 3 viruses mentioned (*Lymphogranuloma venereum* and St. Louis encephalitis) it was noted that better growth was obtained at room temperature (approximately 20-30° C.) than in the incubator at 37° C. In the case of the third virus, that of murine poliomyelitis, the unusual level of potency of 1 to one billion was obtained with the clear supernatant fluid of cultures kept at 37° C.

The present communication deals with the application of the serum-ultrafiltrate technic to the cultivation of the virus of equine encephalomyelitis (Western and Eastern strains).

The virus of equine encephalomyelitis apparently can be grown

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† Fellow in Bacteriology.

1 Sanders, M., *J. Exp. Med.*, 1940, **71**, 113.

2 Molloy, E., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 563.

3 Jungeblut, C. W., and Sanders, M., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 375.