posed clumped chromatin granules. No nucleoli were observed. These nuclear and cytoplasmic features made the cells easily distinguishable from Hofbauer or phagocytic cells.

The specific granules were quite soluble with routine fixation, although some granules were seen streaming out into the villous stroma and apparently dissolving. With the special fixatives the number per cell varied from 10 to 12 to huge numbers densely packed in the cytoplasm, obscuring the nucleus (Fig. 2). These variations may be real or the result of difficulties of fixation. In all material fixed in basic lead acetate the granules were eosinophilic after Wright-Giemsa or azur II-eosin staining and metachromatic after toluidine blue, but remained unstained after methyl green-pyronin.

The constancy of their presence in chorionic villi of placentae of such widely varied stages and their consistent staining qualities after certain fixatives led to the conclusion that these granule-bearing cells may represent an important feature of the histological structure of the chorionic villus. The eosinophilic staining quality and constancy in size and shape of the granules make it highly improbable that they represent phagocytized material. Cells with such metachromatic granules have been reported in the endometrium and myometrium of the bat uterus by Wimsatt (*1*) and by Asplund and Holmgren (*2*) in human uterine mucosa. The latter authors found them only during the fertile period and varying with the stage of the menstrual cycle. They found them rarely during estrogenic stimulation, but in increasing numbers during progestational stimulation, with a premenstrual peak. They apparently disappeared with the menses.

A recent contribution by Pescetto (*3*) describes cells with metachromatic and basophilic granules in placental tissue after 4% basic lead acetate. His figures indicate a marked resemblance to the cells we describe except for the basophilic reaction of the granules he reported and the age range in which he found them.

Although further chemical assay of these granules has not been attempted, it may be pointed out that Sylvén (*4*) in studies of mast cells has demonstrated that heparin is manufactured as a lipoprotein complex in such cells, and that heparin is metachromatic and eosinophilic. It might be suggested that the cellular granules we have described represent heparin or heparinoid substances to be associated with incoagulable, free-flowing blood in such locations where one might assume it would clot. Such granule-bearing cells have been reported only in uterine mucosa (*1, 2*).

The granules we have studied fail to stain with pyronin in Taft's (*5*) method for nuclear protein and are not destroyed by ribonuclease. The special fixers required for their preservation indicate that they are not a mucopolysaccharide.

It is interesting to note that current therapy as used by Rumbolz, Moon, and Novelli (*6*) in certain types of abnormal uterine bleeding involves intravenous introduction of toluidine blue or protamine sulfate, both of which are known to inactivate heparin. The most striking feature of the cytoplasmic granules we have described is the intensity with which they stain with toluidine blue.

We would suggest then that the metachromatic eosinophilic granules consist of heparin or heparinoid substances that normally are related to the function of maintenance of incoagulable blood in the intervillous spaces of the placenta.

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Effect of Salicylic Acid on the Hypoprothrombinemia Induced by Dicumarol in the Dog and the Rat[1]

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Salicylic acid readily induces a hypoprothrombinemia in the rat (*1*) and augments the hypoprothrombinemia induced by Dicumarol[3] (*2*) in the dog. The purpose of this report is to show that under specified experimental conditions, salicylic acid may actually *reduce* the hypoprothrombinemic response of Dicumarol in the dog as well as in the rat, and prolong the survival time of rats receiving the anticoagulant continuously.

The details of the animal experiments and of the modified one-stage prothrombin assay have already been given in detail (*1–4*). In control studies not included here, the prothrombin time of whole plasma (100%) and of the 50, 25, and 6.25% dilutions was measured. As before, the data will be restricted to the prothrombin time of 12.5% plasma (1 part oxalated plasma, 7 parts saline solution) for reasons emphasized in (*5*).

Acetylsalicylic acid[4] will increase and prolong the

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[3] Dicumarol is the trademark for the anticoagulant 3,3′-methylenebis (4-hydroxycoumarin).

[4] Acetylsalicylic acid (aspirin) caused the most drastic hypoprothrombinemia of a large number of salicylate compounds tested in the rat (*6*).

hypoprothrombinemia induced by Dicumarol in the dog (*2*). This effect was readily reproducible when the acetylsalicylic acid was given *after* the anticoagulant and a hypoprothrombinemia had become detectable. This was also true when doses of acetylsalicylic acid up to 100 mg/kg were given simultaneously *with* the anticoagulant (*2*). As noted here for the first time, however, when 1 g/kg of acetylsalicylic acid was given per os *with* the anticoagulant, not only was the hypoprothrombinemic effect of the anticoagulant partially or completely suppressed, but, for several days following, a transitory hyperprothrombinemia could be detected.[5] This response was not obtained solely with the use of huge doses of acetylsalicylic acid. By feeding 10 or 100 mg/kg of the salicylate daily for 7 days prior to the administration of the anticoagulant, a marked reduction in the extent and duration of the hypoprothrombinemia evoked by the anticoagulant alone was observed. Representative results from some thirty trials are given in Table 1.

TABLE 1

REPRESENTATIVE EFFECTS OF FEEDING ACETYLSALICYLIC ACID PRIOR TO, OR SIMULTANEOUSLY WITH, DICUMAROL ON THE HYPOPROTHROMBINEMIA INDUCED BY THE ANTICOAGULANT*

Dose/kg	Manner of feeding acetylsalicylic acid	Prothrombin time (sec) of 12.5% plasma (after giving anticoagulant)							
		Normal	1 day	2 days	3 days	4 days	5 days	6 days	7 days
15 mg Dicumarol		27	39	67	65	63	39		
" " " + 10 mg salicylate in 8 daily doses	Begun 7 days before Dicumarol	25	40	29	23	23			
10 mg Dicumarol		25	51	96	65	38			
" " " + 100 mg salicylate in 8 daily doses	Begun 7 days before Dicumarol	25	77	25	22				
15 mg Dicumarol		27	44	54	37	26			
" " " + 1 g salicylate in 1 dose	Simultaneously with Dicumarol	22	17	19	18	19			
10 mg Dicumarol		25	38	64	71	59	36		
" " " + 1 g salicylate in 2 daily doses	One day before and simultaneously with Dicumarol	22	38	89	24	20	18	17	21
7.5 mg Dicumarol		25	28	69	28	23			
" " " + 1 g salicylate in 3 daily doses	Simultaneously with Dicumarol and for 2 days thereafter	23	17	20	20	20	19	20	22

* Each line of figures is a series of values obtained with a single animal, the test being performed on the same standardized animal.

The effect of acetylsalicylic acid on the action of the anticoagulant was also tested in the rat. When single oral doses of acetylsalicylic acid, 100 mg or more, were given either with Dicumarol (24 hr before sampling) or 12 hr later, the hypoprothrombinemia induced by the anticoagulant alone was reduced (Table 2). Supplementing anticoagulant-fed rats with salicylates also prolonged their survival time. Thus, control rats maintained on the low vitamin K semisynthetic diet survived a daily intake of 2 mg of the anticoagulant for approximately 13 (5–23) days (*3*). When 10 mg acetylsalicylic acid was added to the ration in combination with the anticoagulant, the average survival of 6 rats was 22 (7–58) days. With a daily intake of 80 mg of acetylsalicylic acid the survival time was 19 (9–26) days (6 rats).

A comparative study was made of the hypothrombinemic plasmas obtained from dogs and rats given either Dicumarol or acetylsalicylic acid. The clotting times of the mixed plasma (undiluted, or diluted to 12.5%) were the mean of the two component plasmas, suggesting that the hypoprothrombinemia evoked by the anticoagulant and salicylic acid affect the same or similar fractions of prothrombin (*7*). These studies were previously reported from this laboratory in (*6*).

That 3,3′-methylenebis(4-hydroxycoumarin) in relatively minute, and salicylates in relatively massive, quantities induce a hypoprothrombinemia might have been considered coincidental were it not that the anticoagulant and salicylic acid bear a strong synthetic-degradation relationship *in vitro* (*1*). This finding has sustained the assumption that a similar *in vivo* mechanism operates, although indeed neither has an intermediate compound nor salicylic acid been found in examination of animals given 3,3′-methylenebis(4-hydroxycoumarin) (*6*). More annoying still is the absence of firm evidence pointing to the mechanism whereby both agents affect a hypoprothrombinemia. It is still assumed that there is a suspension of a hepatic synthesis of the protein (*8*).

It has been held that the demonstration of vitamin K inhibition of 3,3′-methylenebis(4-hydroxycoumarin) (*3*) represents a biological antagonism because of a gross similarity in the structure of the two agents producing a competitive phenomenon (*9*). The same phenomenon of competitive inhibition can be drawn upon

[5] In other unpublished experiments, we have observed that compounds chemically related to those possessing anticoagulant properties (various coumarin and salicylate derivatives) induced a detectable *hyperprothrombinemia* in trials where they failed to induce a *hypoprothrombinemia* in rats.

TABLE 2

THE EFFECT OF ACETYLSALICYLIC ACID* ON THE HYPOPROTHROMBINEMIA INDUCED IN THE RAT BY FEEDING 2.5 MG DICUMAROL†

Dose of acetylsalicylic acid (mg)	Prothrombin time (sec) of 12.5% plasma		No. of rats
	Acetylsalicylic acid given 12 hr after anticoagulant	Acetylsalicylic acid given with anticoagulant	
None	116 (86–152)	116 (86–152)	365
10	109 (70–150)	113 (100–125)	12
100	87 (60–160)	101 (98–105)	14
200	88 (78–129)	78 (51– 97)	12
300	89 (78–113)	66 (60– 80)	12

* Given by stomach tube in a suspension with 2% gum tragacanth.

† In the standard procedure of this laboratory (3), blood samples are removed 24 hr following the administration of the anticoagulant.

to explain vitamin K-salicylate antagonism. The *in vivo* detoxication of salicylates is known to produce a variety of degradation and conjugation products (*10*), a mechanism which conceivably could lead to the accumulation of an agent acting as an inhibitor of coumarin hypoprothrombinemia. Similarly, the evolution of a metabolically derived inhibitor would explain the relative difficulty in inducing hypoprothrombinemia with salicylates. It may be suggested that salicylate detoxication results in a derivative which closely resembles the as yet unknown metabolic derivative(s) of 3,3′-methylenebis(4-hydroxycoumarin) causative of hypoprothrombinemia and as a biological antagonist produces inhibition of hypoprothrombinemia. That the salicylate factor can accumulate in sufficient quantity preceding the administration of the anticoagulant may explain why an inhibition results, whereas a superimposed hypoprothrombinemia develops from administration of salicylates *after* the anticoagulant.

That salicylates can be given to rats *after* coumarin anticoagulant hypoprothrombinemia has been established and still reduce its extent militates against the possibility that salicylates may inhibit Dicumarol absorption, and points up the difference in susceptibility of dogs and rats to the hypoprothrombinemia induced by salicylic acid (*1, 2*). The testing of individual metabolic derivatives of the salicylate molecule in animals given Dicumarol has yet to be done and may prove helpful in elucidating the pathway of coumarin anticoagulants.

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Effects of Soils and Sunlight on Dilute Concentrations of Sodium Pentachlorophenate[1]

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In field trials to determine the effects of potential molluscacides on the snail intermediate hosts of *Schistosoma mansoni,* the human blood fluke in Brazil, variable results were occasionally obtained with a given chemical. The probability of soil being one of the factors that influence the efficacy of molluscacides was indicated by observations that the mortality of snails was usually lower in test plots having a muddy bottom. These observations were further supported by tests showing that concentrations of the pentachlorophenate group of molluscacides decreased most rapidly in test plots having a thick underlying layer of soft mud. With the tests developed by Haskins (*1*), microgram quantities of the molluscacides, copper and sodium pentachlorophenates, were easily determined. Unfortunately, analytical methods for determining other molluscacides in high dilutions were not available.

To exclude other factors that may inactivate molluscacides under field conditions, experiments to determine the effect of various soils on sodium pentachlorophenate were carried on in the laboratory. For the tests, different types of water-saturated soil, taken from waters inhabited by planorbid snails, were packed into 1000-ml beakers at the proportions indicated in Table 1. Each beaker was then filled with a solution having a pH of 7.2 and containing 10 ppm sodium pentachlorophenate. Dried mud was used in Expts. 5 and 6, and in the latter it was mixed with the solution until an opaque suspension was formed. A 10-ppm solution of sodium pentachlorophenate served as a control (No. 9). The room temperature during the period of observation ranged from 27° to 33° C.

The laboratory findings corroborate the field observations in that various earthy materials in some way reduced the concentrations of sodium pentachlorophenate. The greater the depth of the mud in proportion to the depth of the water (Expts. 1–4), the more rapid the decrease. Although the mud used in these experiments came from the same place, similar results were obtained with soils from other sites. In preparation of Expt. 5 the water became cloudy with a suspension of mud, which apparently caused the very rapid diminution in concentration of the chemical. Deliberate stirring of the soil suspension (No. 6) resulted in a reduction to 5 ppm in 1 hr, with only a trace of chemical present 4 hr after treatment. In Expts. 7 and 8 the sandy loam and sand with smaller quantities of

[1] From studies conducted at the Instituto Aggeu Magalhães by the Laboratory of Tropical Diseases of the National Institutes of Health, USPHS, under the sponsorship of the Pan American Sanitary Bureau in cooperation with the Divisão de Organisação Sanitaria do Departamento Nacional de Saude of Brazil.