by estradiol in the guinea pig has been compared. Desoxycorticosterone acetate is quantitatively more efficient than testosterone propionate. Desoxycorticosterone is also more efficient than testosterone in antagonizing other effects of estradiol such as opening of the vagina and increase of the uterine weight.

13295

Effect of Vitamin D on Binucleated Cells of the Liver.*

WALTER HEYMANN AND WILLIAM B. WARTMAN.

From the Department of Pediatrics and the Institute of Pathology, Western Reserve University and the University Hospitals of Cleveland.

It has long been known that among the cells of the mammalian liver, there are some which have more than one nucleus. Most of such multinucleated cells contain two nuclei and are thus called binucleated hepatic cells. During fetal life only small numbers of binucleated cells are found, but after birth the number gradually increases until adolescence, after which time, under normal conditions, the number of cells remains constant. Each animal species has a definite percentage of binucleated cells which is equal in the various lobes of the liver and is more or less characteristic of the species.[1] Several investigators have shown that there are more binucleated cells at the periphery of the lobules than at the center.[2, 3] Although there is no conclusive evidence to support the idea, these cells are supposed to result either from hyperactivity and incomplete karyokinesis or from fusion accompanying degeneration.[4]

Szittay[3] reported that the daily administration of 3 cc of Vigantol over a period of 8 days to young rabbits led to an increase in the number of binucleated cells in the periphery of the lobule, although no changes were found in the central zone. The number of cells with 3 and 4 nuclei was slightly increased in the same region, but there was no increase in the number of mitoses. Szittay thought

* Presented before the American Association of Pathologists and Bacteriologists, Pittsburgh, Pa., May 22, 1940.

1 Münzer, F. T., *Arch. f. mikr.-anat. u. Entwcklngsmechn.*, 1923, **98**, 249.

2 Noel, R., *Compt. rend. Soc. de biol.*, 1923, **88**, 212.

3 Szittay, Z., *Compt. rend. Soc. de biol.*, 1937, **124**, 296.

4 Mann, F. C., in Cowdry, E. V., *Special Cytology*, Paul B. Hoeber, Inc., New York, 1932, **1**, 341.

that his findings indicated increased activity in the peripheral zone of the lobule rather than a toxic action of vitamin D upon the liver. These observations are of interest because clinical and experimental studies suggest that the liver plays an important rôle in the action of vitamin D.[5, 6, 7] Since Szittay's experiments seemed to constitute anatomical evidence of the action of vitamin D on the liver it was decided to repeat them.

In the first series of experiments the work of Szittay was repeated using young rats, dogs and rabbits. The rats were maintained on the Sherman diet B as modified by Smith and Bing,[8] which was supplemented with yeast and wheat germ oil. The dogs were fed Purina dog chow. The rabbits were given cabbage and carrots, which was the diet used by Szittay for his animals. All animals were allowed as much water as they would drink.

The animals of each species were divided into several groups. In the first group the rats received 1 cc and the rabbits and puppies 3 cc of viosterol. In the second group the rats received 1 cc and the rabbits 3 cc of Wesson oil. The third group consisted only of rabbits and puppies which received 3 cc of sesame oil. These substances were administered daily to the rats by pipette and to the rabbits and puppies by stomach tube. The fourth group was given thyroxin by intramuscular injection. The fifth group was used as a control and received nothing but the diet. Samples of blood were drawn before each experiment as well as at the conclusion and were analyzed for calcium and phosphorus. The animals were killed either by decapitation or intravenous injection of air, and thin slices of all organs were removed immediately, fixed in neutral 10% formalin and Zenker's fluid, embedded in paraffin, sections cut at 6 micra and stained with hematoxylin and eosin. At least 200 cells were counted in the central zone and another 200 cells in the peripheral zone of the liver lobule in each of the animals. The results were expressed as the mean number of binucleated cells per 100 liver cells.

Sesame and Wesson oils were used as controls to determine the effect of simple oils upon the liver. Thyroxin was used because it was thought that the increase in the number of binucleated cells might be due to the increase in metabolism which is known to occur with large doses of vitamin D.

Results. In young rats and dogs there was no increase in binu

[5] Gerstenberger, H. J., *Monatschr. f. Kinderh.*, 1933, **56**, 217.

[6] Gerstenberger, H. J., cited in Griffith, J. P. C., and Mitchell, A. G., *Textbook of Pediatrics*, Philadelphia, W. B. Saunders Co., ed. 3, p. 549, 1941.

[7] Heymann, W., *Am. J. Dis. Child.*, 1938, **55**, 913.

[8] Smith, A. H., and Bing, F. J., *J. Nutrition*, 1928, **1**, 179.

cleated cells in either the central or peripheral zones of the lobule following the administration of vitamin D or of the control substances.

In rabbits, however, changes similar to those described by Szittay were observed (Table I). After the fourth day of administration of viosterol the number of binucleated cells in the peripheral zones of the lobules was definitely increased. It should be emphasized that the increase occurred only in the peripheral portion of the lobule and was never observed in the central zone where the number of binucleated cells remained stationary at an average of 10 per 100 liver cells. Hence these data have not been put in the table. Control animals which received either no medication or plain sesame and Wesson oils did not show a rise in the number of binucleated cells. Likewise the rabbits which received thyroxin failed to show this change, indicating that even if the basal metabolism of the animals was elevated, it did not cause the increase in binucleated cells.

In order to determine whether or not vitamin D was the specific cause of this phenomenon, the effect of inactivated viosterol was studied. Viosterol was exposed for 98 hours to a mercury vapor quartz light at a distance of one foot. This over-irradiated viosterol was tested in rachitic rats in the usual manner and found to be completely inactivated. When the inactivated viosterol was administered to rabbits by stomach tube, in doses of 3 cc, the same result was obtained as when potent viosterol was used. Because this was an unexpected result and because viosterol is not a solution of pure vitamin D_2, the experiments were repeated, using Drisdol, which is a solution of crystalline vitamin D_2 in propylene glycol. Again the results were surprising. The daily administration of 3 cc of Drisdol, or a similar dose of Drisdol which had been inactivated by over-irradiation with ultraviolet light for 120 hours, as well as the solvent propylene glycol, led to a definite increase in the number of multinucleated cells in the peripheral zones of the lobules.

No correlation was found in any of the experiments between an increase in organic phosphates in the blood serum and an increase in binucleated cells in the peripheral zone of the lobule. For example, hyperphosphatemia occurred in rats and dogs fed viosterol, but the binucleated cells did not increase. On the contrary, the administration of inactivated viosterol and of Drisdol did not cause hyperphosphatemia in rabbits, but did result in an increase in the number of binucleated cells. These results proved beyond doubt that the phenomenon was not caused by vitamin D itself.

Because vitamin D, inactivated vitamin D and propylene glycol are chemically alcohols, preliminary experiments were made by

TABLE I.

Animal	Amount of drug administered	Duration of experiment days	% of binucleated cells in periphery of liver lobule
Rabbit			
16	Viosterol 3 cc	4	27
17	" "	6	65
18	" "	8	58
6	" "	8	43
33	" "	12	53
7	" "	14	45
25	" "	15	58
37	Irrad. Viosterol 3 cc	6	40
31	" " "	12	61
1	Wesson Oil 3 cc	8	27
27	Thyroxin 0.2 mg	8	21
29	" 0.3 "	8	20
28	" 0.3 "	9	25
38	Drisdol 3 cc	8	49
39	" "	15	73
42	Irrad. Drisdol 3 cc	8	54
43	" " "	10	21
15	Sesame Oil 3 cc	8	28
36	" " "	14	13
14	" " "	15	26
40	Propylene Glycol 3 cc	6	68
41	" " "	15	46
55	Ethyl Alcohol 10 cc	1	42
54	" " 5 "	4	22
60	" " 10 "	4	26
57	Ether 2 cc	1	44
56	" 1 "	2	45
47	" 3 "	1	26
48	" 3 "	2	24
13	Control	15	25
50	"	15	23
51	"	15	20

feeding absolute alcohol and ethyl ether (Squibb) to rabbits. As seen in the table, both alcohol and ethyl ether caused an increase in the number of binucleated cells in the livers of some, but not all, of the animals treated. Thus it would appear that although these substances can, they do not constantly, bring about the increase, and further that the cellular change is not specific for alcohols.

Summary. It has been shown that the increase in binucleated

cells in the peripheral zone of the lobule of the liver which Szittay observed after the feeding of Vigantol to young rabbits is not due to a specific action of vitamin D as he believed. The increase also occurs after the administration of inactivated viosterol, Drisdol, inactivated Drisdol, propylene glycol, absolute alcohol and ethyl ether.

The fact that young rats and dogs do not show this phenomenon, even though vitamin D is known to be just as effective against rickets in these animals as in rabbits, suggests that there is a species difference.

There is no detectable relationship between the effect of vitamin D on the phosphorus and calcium content of the blood and the increase of multinucleated cells in the liver. It is unlikely that an increase in basal metabolism is responsible for the phenomenon since the administration of thyroxin does not lead to an increase in the number of multinucleated cells. Wesson oil and sesame oil are without effect.

The observation that ethyl ether as well as propylene glycol and absolute alcohol may bring about an increase in the number of multinucleated cells suggests that a number of chemically different substances may be responsible for the increase.

13296 P

Hemorrhage Control in *Eimeria tenella* Infected Chicks When Protected by Anti-Hemorrhagic Factor, Vitamin K.

FRANCIS MARSH BALDWIN, OZRO BRANDT WISWELL AND HARRY ANTHONY JANKIEWICZ.

From the Physiological Laboratories, The University of Southern California, Los Angeles.

Excessive amounts of vitamin K in the dietary are usually considered a necessary factor in cases where prothrombin levels must be built up. However, as far as the authors are aware there has been no work on the control of hemorrhage in cases of parasitism by the coccidium, *Eimeria tenella,* in chicks in relation to the anti-hemorrhagic vitamin K. This vitamin was obtained in the liquid form, a peanut oil compound, under the trade name, "Klotogen".* A pure culture of *E. tenella* was obtained from the Los Angeles

* Through courtesy of Abbott and Company, Los Angeles, California. (Each cc of this liquid vitamin is equivalent to 1250 Almquist units, or 46,500 Dam units.)