able, this aspect should be investigated. It has been suggested that toxemia of pregnancy is similar in a number of respects to the syndrome of DOCA intoxication(10). It also has been found that the sodium-retaining activity of urine is increased during late pregnancy with the greatest increase found in toxemia of pregnancy(11). These findings appear to lend support to the aforementioned suggestion.

It was not feasible to study the ability of the maternal organism to utilize exogenous cortisol after delivery. Hence, no estimate can be made of the significance of the per cent of exogenous cortisol remaining in the plasma at a given time, except to say that the maternal organism has the ability to use an amount of cortisol greatly in excess of the increment found in 17-OHCS in pregnancy. It would appear, then, that an increased rate of production, rather than a decreased rate of utilization, is responsible for much, or all, of the elevated level of 17-OHCS seen in pregnancy. It is recognized that the data presented herewith do not rule out the possibility that the placenta and, or, the fetal adrenals may contribute to the elevated level of plasma 17-OHCS.

Summary. Adrenal cortical capacity and cortisol utilization were studied in 20 patients with normal pregnancy and 19 patients with toxemia of pregnancy. It was found that the level of plasma 17-OHCS in late pregnancy can be further elevated by the action of exogenous ACTH. The reserve capacity of the adrenal cortex in normal pregnancy is similar to that in non-pregnant subjects; in toxemia of pregnancy it is somewhat reduced. Utilization of infused cortisol in both normal and toxemic pregnancies does not appear to be faulty; thus utilization does not seem to be a factor in the elevated levels of plasma 17-OHCS found in pregnancy.

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The *in vitro* and *in vivo* Effect of Synkavite on *Histoplasma capsulatum*.* (22624)

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De Saint-Rat and Luteraan(1) demonstrated that 5-hydroxy-2-methyl-1, 4-naphthoquinone in concentrations of 1:50,000 was active, *in vitro*, against *H. capsulatum*. Synkayvite, Roche (tetrasodium 2-methyl-1, 4-naphthohydroquinone diphosphoric acid ester), is a synthetic water-soluble compound of similar basic structure, potent vit. K activity, and relatively low toxicity. These characteristics, together with stability to heat and easy availability, resulted in the selection of this drug for this study.

It was the purpose of the following experi-

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ments to test the potency of Synkayvite as an inhibitory agent against yeast-phase *H. capsulatum, in vitro*, and as a potential chemotherapeutic agent in experimental histoplasmosis in mice. In addition, studies were performed to evaluate the *in vitro* effect of this drug against certain other fungi, pathogenic and nonpathogenic to man.

Materials and methods. The sensitivity of 8 fungi, (Table I), to Synkayvite was determined on brain-heart infusion agar slants containing the following concentrations of Synkayvite in mg per ml: 4.6, 2.3, 1.2, 0.6, 0.3, 0.15, 0.08, 0.04, 0.02 or 0.01. Care was taken to prevent exposure of the Synkayvite to light, and the slants were incubated for 48 hours to test for bacterial contamination. Control media containing no drug were prepared along with an additional control group containing 0.56, 0.28, 0.14, 0.07, 0.04, 0.02, 0.01 or 0.005 mg of phenol per ml of media to correspond with the amount of phenol present as a preservative in the 8 highest concentrations of Synkayvite listed above. All slants were inoculated with 0.3 ml from 3-week-old broth cultures of all of the fungi except *H. capsulatum* where 0.5 ml saline-yeast-phase suspensions from 3-day-old brain-heart infusion agar slants were employed. The inoculated tubes were sealed with parafilm and incubated in the slanted position at optimal temperatures for a period of 1 month. The amount of growth was recorded as 1 to 4 plus or negative, as compared to controls containing no Synkayvite or phenol, and the minimal inhibitory concentrations of both Synkayvite or phenol noted.

Toxicity studies of Synkayvite for 6-week-old normal white male Swiss mice were divided into 2 parts. Part 1 was designed to determine the toxicity of a single intraperitoneal or subcutaneous injection of 0.2 ml of saline containing 0.3 to 0.55 mg of naphthoquinone per g of mouse. Controls received .07 mg of phenol per g in 0.2 ml of saline, a dose comparable to that contained in 0.55 mg of Synkayvite. The second toxicity experiment was designed to test the maximum daily dose of Synkayvite (from .025 to 0.2 mg/g of mouse) which could be tolerated up to 28 consecutive days. Two groups of controls were used: one received phenol in 0.2 ml of saline in concentrations analogous to those contained in the corresponding Synkayvite doses (Table III); the second received 0.2 ml of saline only, daily. The number of deaths and gain or loss of weight of animals in each group were recorded at the end of 28 days.

TABLE I. *In Vitro* Inhibitory Effect of Synkayvite and Phenol on Fungi.

Fungus	Synkayvite: Minimum conc.* for complete inhibition	Synkayvite: Phenol conc.* present	Phenol controls: Conc.* for partial inhibition
H. capsulatum	.15	.01	.56
N. asteroides	.60	.07	.56
N. braziliensis	.30	.04	.56
T. mentagraphyta	.60	.07	1.12
M. canis	1.20	.14	1.12
E. floccosum	.30	.04	1.12
Aspergillus fumigatus	.30	.04	.28
Scopulariopsis sp.	.30	.04	1.12

* Concentration in mg/ml of brain-heart infusion agar.

The effect of Synkayvite therapy in the mice infected with *H. capsulatum* was tested following intraperitoneal inoculation with 35 million yeast-phase organisms in 0.5 ml of mucin(2). At intervals ranging from 3 days to 10 weeks after histoplasma inoculation, daily intraperitoneal injections of Synkayvite were given up to 28 days, whenever possible. All doses of the naphthaquinone were administered in 0.2 ml of saline, and control animals received the greatest amount of phenol in 0.2 ml of saline received by test animals (Table IV).

Results. All of the fungi tested were inhibited completely, *in vitro*, by concentrations of 0.15 to 1.2 mg/ml of Synkayvite (Table I). That the amount of phenol present in these quantities of Synkayvite was not responsible was demonstrated by the fact that considerably more phenol than was present in the Synkayvite was required for even partial inhibition of growth. These observations were particularly striking with *H. capsulatum* in which only 0.15 mg of the naphthaquinone containing only .01 mg of phenol caused complete inhibition of growth while 0.56 mg of phenol alone did not completely inhibit

TABLE II. Effect of Single Doses of Synkayvite in Normal Mice.

Intraper. inoc.			Subcut. inoc.		
Avg wt (g)	Sk. dose*	No. deaths‡	Avg wt (g)	Sk. dose*	No. deaths‡
15	.30	0/6	16	.30	0/4
16	.35	0/6	15	.35	0/4
15	.40	0/6	17	.40	0/4
15	.45	2/6	16	.45	2/4
17	.50	6/6	16	.50	4/4
15	.55	6/6	15	.55	4/4
16	Phenol control†	0/6	17	Phenol control†	0/4

* Sk. dose: Synkayvite in mg/g of animal.
† Control animals received .07 mg of phenol in .2 ml saline/g of mouse.
‡ Numerator = No. of deaths. Denominator = No. of mice.

growth.

Acute toxicity studies in mice revealed that toxic doses of Synkayvite resulted in peripheral vasodilatation, cyanosis, shock, and death 20 minutes to 4 hours after either subcutaneous or peritoneal inoculation. Table II demonstrates that normal mice could tolerate single doses up to 0.40 mg/g weight. No untoward effect was noted in mice receiving .07 mg of phenol per g, an amount equivalent to that contained in 0.6 mg of Synkayvite. Chronic toxicity studies revealed that the mice could tolerate up to 0.15 mg of the naphthaquinone per g of body weight, daily, for 28 days (Table III). When 0.2 mg per g containing .024 mg/g of phenol was used 3 of 10 mice died as did 4 of 10 receiving .024 mg of phenol, alone.

When infected mice were given Synkayvite in doses of .05 to 0.2 mg per g of weight,

TABLE III. Effect of Multiple Intraperitoneal Doses of Synkayvite in Normal Mice.

Daily dose (mg)		Avg wt (g)	Avg wt change after 28 days (g)	No. deaths at 28 days§
Sk.*	Phenol†			
.20	.024	17.4	−2.1	3/10
.15	.018	17.0	− .2	0/10
.10	.012	18.1	+1.0	1/10
.05	.006	17.8	− .8	0/10
.025	.003	16.9	+2.1	0/10
.0‡	.024‡	17.7	−3.3	4/10
.0‡	.003‡	16.4	+1.3	0/10

* Expressed as mg/g of mouse.
† As contained in the Synkayvite.
‡ Phenol controls.
§ Numerator = total No. mice. Denominator = total No. deaths.

between the 3rd to 21st day after inoculation with *H. capsulatum,* a markedly accelerated death rate was observed (Table IV). Death would occur within 20 to 240 minutes after a dose of Synkayvite, with signs of shock similar to those observed in the acute toxicity studies above (Group A, Table IV). In mice receiving 0.2 mg/g 3 days after infection, 8 of 10 died after 1 to 3 doses, or within 6 days after histoplasma inoculation. By the 9th day of infection, or after 6 daily doses of 0.2 or 0.1 mg of Synkayvite, 100% mortality was observed, while 6 of 9 receiving .05 mg died. None of the 6 infected controls receiving .024 mg of phenol, the amount equivalent to that contained in 0.2 mg of Synkayvite, and only 16 of 47 (34.4%) untreated controls (Group G) died during this interval.

When therapy was started 7 or 14 days after infection (Table IV, Groups B, C) increased susceptibility to Synkayvite was more marked in that practically 100% mortalities followed 1 to 3 injections of 0.2, 0.1, or .05 mg. This time interval of 7 to 14 days represented the peak death rate in the untreated controls(2-5) (Group G) where 35 of 47 died of specific infection during the interval. However, the rapid fulminating shock-like type of death was noted only in those mice receiving Synkayvite. This rapidly lethal effect was still evident in mice given Synkayvite which still survived 21 days after infection (Group D, Table IV). However, mice surviving 28 days after infection required 7 to 28 injections before this shock-like syndrome could be induced. When mice which had survived infection induced 10 weeks previously were given up to 28 daily injections of Synkayvite, only 1 of 25 reacted unfavorably to the drug (Group F, Table IV). As can be seen through Table IV, mice receiving .024 mg of phenol per g reacted in a manner similar to untreated infected controls.

Discussion. Alcohol-soluble quinones and quinolines have been known to possess antifungal properties and this subject has been reviewed recently by Oster and Golden(6). These studies demonstrate that tetrasodium 2-methyl-1, 4-naphthohydroquinone disphosphoric acid ester in concentrations of 0.6 mg/

TABLE IV. Effect of Synkayvite on Fatality Rate of Mice Infected with 35 Million Yeast-Phase *H. capsulatum*.

Group	No.*	Post inoc., time therapy started	Daily dose, mg/g†	Deaths during 3-day periods after 1st dose 1–3	4–6	7–10	11–14	15–28	% fatalities in 28 days‡
A	10	3 days	.20 sk	8	2				100
	13		.10 "	4	9				100
	9		.05 "	4	2	3			100
	6		.024 ph	0	0	4	1	1	100
B	8	7 "	.20 sk	8					100
	9		.10 "	9					100
	9		.05 "	8	1				100
	6		.024 ph	1	3	1	0	1	100
C	8	2 wk	.20 sk	8					100
	7		.10 "	7					100
	7		.05 "	7					100
	6		.024 ph	0	2	0	0	1	50
D	7	3 "	.20 sk	7					100
	7		.10 "	7					100
	7		.05 "	6	1				100
	6		.024 ph	1	1	0	0	0	33
E	8	4 "	.20 sk	1	0	2	2	2	88
	8		.10 "	0	0	1	0	4	63
	8		.05 "	0	0	0	0	3	38
	6		.024 ph	0	0	1	0	0	17
F	9	10 "	.20 sk	0	0	0	1	0	11
	8		.10 "	0	0	0	0	0	0
	8		.05 "	0	0	0	0	0	0
	6		.024 ph	0	0	0	0	1	17
G	47	Control	None	0	3	13	19	7	89§

* No. of mice chosen from surviving animals for each therapeutic group.

† sk = daily dose of Synkayvite containing phenol; ph = daily dose of phenol equivalent to amt received by test animals receiving highest amt of Synkayvite.

‡ Fatalities occurring in 28 days after therapy was started.

§ None of remaining mice died after 28 days; death from specific infection rarely occurs 30 days post inoculation(2-5).

ml or less will inhibit the growth of 7 of 8 potentially pathogenic fungi tested while 1.2 mg will inhibit the 8th. This effect was particularly evident against *H. capsulatum* where 0.15 mg/ml of medium caused complete inhibition. However, this promising aspect was not evident during *in vivo* studies. In fact, death rates were actually accelerated when previously infected mice received intraperitoneal injection of Synkayvite. Death would occur within 20 to 240 minutes after administration of naphthaquinone with shock-like signs similar to those observed in non-infected mice receiving toxic doses of drug. This effect was particularly prominent during the 7 to 21-day post-infection time interval when peak deaths from specific infection occurred in untreated controls, but was not apparent when mice which had survived infection 10 weeks previously received 28 daily injections of drug. Thus, it is suggested that the inherent toxic properties of Synkayvite are potentiated during the period of acute infection. Since histoplasmosis is a reticulo-endothelial disease and could alter liver function in a patient with disseminated disease, the question arises as to whether the administration of Synkayvite as a vit. K preparation could possibly aggravate the disease process. These results suggest that close observation for any toxic reactions be instituted in any patient with acute histoplasmosis who should, by any chance, receive a naphthoquinone medication.

Conclusions. 1. Tetrasodium 2 methyl-1, 4-naphthohydroquinone disphosphoric acid ester in concentrations of 0.15 mg/ml inhibited completely the *in vitro* growth of *H. capsulatum* while 0.3 to 0.6 mg/ml caused complete inhibition of *Nocardia asteroides, N.*

braziliensis, Trichophyton mentagrophytes, Epidermophyton floccosum, Aspergillus fumigatus, and a species of *Scopulariopsis. Microsporon canis* required 1.2 mg/ml for complete inhibition. 2. Six-week-old white mice could tolerate a single intraperitoneal or subcutaneous inoculation of Synkayvite up to 0.4 mg/g of weight but not 0.45 mg. Mice could also tolerate up to 0.15 mg/g daily for 28 days. 3. Mice infected with *H. capsulatum* could not tolerate non-toxic doses (to normal mice) of Synkayvite. This effect was particularly apparent between 7 to 21 days, but not 10 weeks, after infection. 4. The possibility that this type of medication in acute histoplasmosis could aggravate the disease is suggested.

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Importance of Anemia in Artificially Induced Metabolic Acidosis. (22625)

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It has been established that the plasma carbonic acid-sodium bicarbonate buffer group is the important buffer system in overcoming metabolic acidosis induced by accumulation of organic and inorganic acids. On the other hand, while the erythrocyte with its hemoglobin buffers as much as 92% of the endogenous carbonic acid, its effectiveness against fixed acids is uncertain.

In a given clinical situation acidosis and anemia may co-exist as in the anemic phase of glomerulonephritis, an occasional case of diabetic acidosis and the late stage of pyelonephritis. Bland(1) feels that hemoglobin as administered by transfusion of whole blood or red cell mass is fully as important a buffer as bicarbonate. This study was undertaken to determine whether the presence of anemia in itself had any adverse effect on the body's ability to overcome an artificially induced metabolic acidosis.

Materials and methods. Three volunteer patients suffering from severe blood loss anemia due to bleeding peptic ulcer were studied. These patients had no evidence of cardiac, pulmonary, or renal disease. All tests were performed in the postabsorptive state. At 8:00 a. m. the morning of the test, the bladder was catheterized and the catheter left indwelling. Initial blood serum bicarbonate, chloride, sodium, potassium, non-protein nitrogen, total and fractional proteins, and pH (Beckman pH meter) were determined along with red blood count and hemoglobin determination. (Sahli). An aqueous ammonium chloride* solution was infused intravenously at a constant rate so that 200 mEq. of ammonium chloride would be administered in exactly a 4-hour period.

At hourly intervals for the next 8 hours venous blood samples were obtained for pH and bicarbonate determination. At the same time the urinary catheter was opened and the urine collected under oil for determination of pH and total titratable acidity using 0.1 N NaOH and phenol red as an indicator. The pH of whole blood was determined at 25°C by transfer of the sample from a heparinized 5 cc syringe to the Beckman electrode cup and

* Ammonium chloride 400 mEq./1., furnished through the courtesy of Abbott Laboratories, North Chicago, Ill.