TABLE I.
Blood and Spinal Fluid Sugar Levels After Intracisternal Injection of Glucose in Man.

Subject	Glucose injected into cisterna mg	Spinal fluid* sugar—mg %				
		Before	15 min.	30 min.	45 min.	60 min.
1	250	62	70	63	64	61
2	250	76				
3	400	57	1011	913	832	630
4	500	60	239	406	761	830
5	500	60	118	80	76	126
6	500	66	65	70	84	97
7	500	72	536	736	782	736
8	500	56	208	638	742	767
		Blood sugar—mg %				
		Before	15 min.	30 min.	45 min	60 min.
1	250	85	82	83	81	76
2	250	88	96	94	97	104
3	400	72	68	70		71
4	500	76	76	71	73	74
5	500	86	90	88	86	86
6	500	79	85	83	82	82
7	500	98	95	100	97	100
8	500	80	85	91	98	81

* Drawn from L4.

also be noted that the intracisternal injection of glucose was followed by a marked rise within 15 minutes in the glucose content of spinal fluid withdrawn from the lumbar region. Such a finding indicates the free diffusion of the injected glucose and may be taken as presumptive evidence that every part of the central nervous system was in contact with an increased concentration of glucose.

Since similar results have been obtained in subjects free from neurologic and psychiatric disorders, it is obvious that an increase in the glucose level of the cerebrospinal fluid does not initiate a chain of reactions resulting in a decrease of the blood sugar level. It seems fairly definite, therefore, that any mechanisms that may be involved in the regulation of carbohydrate metabolism by the central nervous system are not significantly influenced by increase in the glucose content of the spinal fluid.

Summary and Conclusion. Increases in the spinal fluid glucose concentration do not produce any effect on the blood sugar level.

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Infectivity of Sporozoites of *Plasmodium Cathemerium 3H2* Exposed *In Vitro* to Hen and Canary Bloods.*

HARRY BECKMAN.

From the Department of Pharmacology, Marquette University School of Medicine, Milwaukee.

In this laboratory the avian malarial parasite, *Plasmodium cathemerium* 3H2, has been maintained by continuous mosquito-canary-mosquito passage since February, 1937. During all of this time there has never been a failure to infect a canary; *i. e.*, in the rare instances in which a first trial has not succeeded a second trial has always done so. But

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it has not been possible to infect with this organism the great horned owl,[1] the guinea pig,[2] the barnyard hen, or man.[3] In this present communication there will be recorded the results of an attempt to determine whether the blood of the hen contains a factor inhibitory to the sporozoites of *P. cathemerium* 3H2 when they are exposed to it *in vitro*. For the purposes of the study the sporozoites were incubated separately but simultaneously for ½ to 2½ hours in hen and canary bloods, and then the blood-sporozoite mixtures were injected intramuscularly into susceptible canaries.

Method. Using a tuberculin syringe to which was attached a 24-gauge ¾-inch needle filled with 1% sodium heparin solution preserved with 0.5% phenol, 0.2 cc of blood was drawn from the heart of each of 8 canaries to make a total of 1.6 cc. The blood was pooled as drawn in a rubber-stoppered 5-cc vaccine bottle held in the mouth of the operator and kept in motion by a frequent bobbing of the head, both movement of the blood and fair maintenance of temperature being accomplished by this maneuver. The blood was drawn in about 10 minutes total elapsed time and the bottle was then immediately placed in an incubator at 41.5°C, being affixed in the incubator to a rotor making 1 complete revolution per minute. Immediately after bleeding the canaries, an amount of blood—7 cc—thought to be equivalent to the fraction of the total drawn from the canary was taken by heart puncture from each of 3 hens, using a 3-inch "Bishop B-19" needle containing heparin solution in amount proportionate to that employed in bleeding the canaries. At once, 0.5 cc of each of 2 of these hen blood samples and 0.6 cc of the third were placed together in a 5-cc vaccine bottle, making a total of 1.6 cc as in the case of the canary blood. The pool of hen blood was usually prepared and in the incubator rotor in 5 to 8 minutes. Then as quickly as possible the sporozoites were prepared for addition to the bloods, as follows: 100 *Culex pipiens* mosquitoes that had taken an infective blood meal 2 weeks previously were stunned by rapping the Kjeldahl flask in which they were contained a number of times against the palm of the hand, after which they were dumped into a small evaporating dish containing 1.0 cc of Locke's solution. With a fairly large pestle the mosquitoes were then ground for precisely 30 seconds; the triturate was then transferred to 3 layers of gauze in a small funnel protruding through a stopper into a calibrated centrifuge tube and washed through the gauze with sufficient Locke's solution to bring the filtrate up to the 1.0 cc mark in the tube. Then the bottles of blood were taken out of the incubator and 0.4 cc of the sporozoite-containing filtrate, withdrawn from the centrifuge tube through a specially devised long needle with a square end and a very fine bore, was added to each. The bottles, which now contained 2 cc of a sporozoite-blood mixture, were at once returned to the incubator and rotation resumed. Thereafter, at 30-minute intervals through 2½ hours, the bottles were taken from the incubator just long enough to withdraw 0.2 cc of the contents of each into separate tuberculin syringes, having 27-gauge ½-inch needles attached. At each withdrawal period 4 clean canaries were injected intramuscularly with the canary blood-sporozoite mixture and 4 with the hen blood-sporozoite mixture; each bird received 0.05 cc, an amount calculated to contain the sporozoites from 1 mosquito. The peripheral blood of the injected canaries was examined for the presence of plasmodia on the 6th to 16th days, inclusive, following the injections, and thereafter any canaries in whose blood organisms had not appeared were challenged by the bites of infected mosquitoes to determine whether or not they were still susceptible to malarial infection.

Results. Tables I and II comprise the findings in this study. The 4 separate trials were made a sufficient number of weeks apart to permit a full cycle of events to have transpired in the insectory so that each trial was done with a new and freshly infected lot of mosquitoes. In Table I there is ex-

[1] Beckman, H., and Ota, R. K., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 477.

[2] Beckman, H., and Smith, J., unpublished findings.

[3] Beckman, H., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 401.

TABLE I.

Infectivity of Sporozoites of *P. cathemerium* 3H2 Exposed *in vitro* to Hen and Canary Bloods. (Data from a Single Trial.)

Exposure period (hr)	Type of blood	Bird No.	First day of positive blood	Type of blood	Bird No.	First day of positive blood	Result of mosquito challenge
½	canary	1605	9	hen	1497	None	Infected
	,,	1126	9	,,	1665	,,	,,
	,,	1843	8	,,	1983	,,	,,
	,,	1821	8	,,	1777	,,	,,
1	,,	1579	11	,,	822	,,	,,
	,,	1171	10	,,	817	,,	,,
	,,	1482	10	,,	1141	,,	,,
	,,	69	8	,,	1024	,,	,,
1½	,,	1827	10	,,	1856	,,	,,
	,,	1679	10	,,	801	,,	,,
	,,	1637	8	,,	850	,,	,,
	,,	1713	9	,,	961	,,	,,
2	,,	91	10	,,	840	,,	,,
	,,	1086	12	,,	1073	,,	,,
	,,	1642	9	,,	1956	,,	,,
	,,	818	11	,,	878	,,	,,
2½	,,	61	9	,,	1563	,,	,,
	,,	1144	13	,,	1096*		
	,,	1604	10	,,	1555	,,	,,
	,,	831	11	,,	76	,,	,,
Totals:	Birds injected	20		Birds injected	19		All infected
	,, infected	20		,, infected	0		

* Traumatic death on day of injection.

hibited the data from a single trial; it will be noted that organisms were found in the peripheral blood in 8 to 13 days in all of the birds injected with sporozoites in canary blood and that no organisms were found at any time in those injected with sporozoites in hen blood; it will be further observed that all of the latter group of birds did reveal organisms in the peripheral blood when they were subsequently bitten by infected mosquitoes. In Table II, comprising a summary of all the trials, it will be seen that of the 80 birds injected with sporozoites in canary blood 97.5% became infected, while of the 76 birds injected with sporozoites in hen blood only 2.6% became infected; it will also be noted that all 74 of the latter group of birds became infected when subsequently challenged by infected mosquitoes.

Discussion. The findings in the present study establish the fact that the blood of the hen, an animal not susceptible to infection with *P. cathemerium* 3H2, exerts an inhibiting action upon the sporozites of this organism *in vitro*. Coggeshall,[4] stating that there is considerable experimental evidence in support of the assumption that an immune serum can act upon a parasite without the participation of the cells of the host, showed that some sera from monkeys that had survived a *P. knowlesi* infection completely inhibited the infectiousness of the erythrocytic form of the parasite when it was incubated with them for ½ hour. He showed that the protective antibodies in such a serum can be removed by absorbing them by the addition of living parasites and then removing both by centrifugation, but his experimental arrangement did not lend itself to a study of the effect of these sera upon sporozoites. It seems from the work of Boyd[5] that the immune mechanism engendered by an attack of vivax malaria in the human is specifically directed toward the trophozoites

[4] Coggeshall, L. T., *Medicine*, 1943, **22**, 87.

[5] Boyd, M. F., *J. Nat. Mal. Soc.*, 1947, **6**, 12.

TABLE II.
Infectivity of Sporozoites of *P. cathemerium* 3H2 Exposed *in vitro* to Hen and Canary Bloods.
(Summary of all trials.)

	Canary blood		Hen blood		
Trial No.	Birds injected	Birds infected	Birds injected	Birds infected	Result of mosquito challenge
		One-half Hour Exposure.			
1	4	4	2*	0	2 infected
2	4	4	4	0	4 "
3	4	4	4	0	4 "
4	4	4	3*	2	1 "
		One Hour Exposure.			
1	4	3	4	0	4 "
2	4	4	4	0	4 "
3	4	4	4	0	4 "
4	4	4	4	0	4 "
		One and One-half Hours Exposure.			
1	4	4	4	0	4 "
2	4	4	4	0	4 "
3	4	4	4	0	4 "
4	4	4	4	0	4 "
		Two Hours Exposure.			
1	4	4	4	0	4 "
2	4	4	4	0	4 "
3	4	3	4	0	4 "
4	4	4	4	0	4 "
		Two and One-half Hours Exposure.			
1	4	4	4	0	4 "
2	4	4	3*	0	3 "
3	4	4	4	0	4 "
4	4	4	4	0	4 "
Totals	80	78	76	2	74 "

Percentage infected after exposure to canary blood 97.5
" " " " " hen " 2.6

* Numbers less than 4 reflect early deaths from injection trauma.

(*i. e.*, the erythrocyte phase) and not toward the sporozoites. Mulligan, Russell and Mohan[6] reported that the sera of many species of normal animals and man agglutinate the sporozoites of *P. gallinaceum* in low dilutions but that agglutination in high dilutions occurred with only the sera of animals or men having chronic malarious infections. It was not determined in the present study whether the effect of the hen's blood upon the sporozoites was an agglutinative one. Huff and Coulston,[7] failing to find cryptozoites in canaries inoculated with the sporozoites of *P. gallinaceum*, made the assumption that the barrier must exist in macrophages and other phagocytic cells or in antagonistic humoral substances. Since in the present study it is shown that canaries injected with sporozoites that have been exposed to hen blood not only fail to become infected but can later be infected by sporozoites that have not had such contact, it seems likely that hen's blood depresses the sporozoites sufficiently to prevent the initiation by them of the exoerythrocytic cycle.

Summary. Canaries were injected intramuscularly with sporozoites of *P. cathemerium* 3H2 in pooled hen or canary blood, the sporozoite-blood mixtures having been incubated previously at 41.5°C, with agitation, for periods of ½, 1, 1½, 2, and 2½ hours. Of 80 canaries injected with sporozoites in canary blood, 97.5% became infected; of 76 canaries injected with sporozoites in hen blood, 2.6% became infected. All 74 canaries which failed to become infected when injected with

[6] Mulligan, H. W., Russell, P. F., and Mohan, B. N., *J. Mal. Inst. India*, 1940, **3**, 513.

[7] Huff, C. G., and Coulston, F., *J. Infect. Dis.*, 1946, **78**, 99.

sporozoites in hen blood did become infected when subsequently bitten by infected mosquitoes. It is felt that these findings suggest the existence of a factor in the blood of the hen that prevents the initiation by the sporozoite of the exoerythrocytic cycle in the canary.

Subsequent note: Further studies, completed after the above manuscript was submitted for publication, have revealed that (a) the inhibitory action of hen's blood on the sporozoites is exerted though sodium citrate is used as anticoagulant instead of heparin; and (b) it is not lessened by agitating the blood at room temperature for an hour before adding the sporozoites to it.

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Activity of Hexokinase Preparations from Rat Muscle.*

ROBERT H. BROH-KAHN AND I. ARTHUR MIRSKY.
(With the technical assistance of Suzanne Claugus.)
From the May Institute for Medical Research, The Jewish Hospital, Cincinnati, Ohio.

Hexokinase activity involves the conversion of an easily hydrolyzable phosphate group to one not easily hydrolyzable (glucose-6-phosphate). Since the P_7† value of the reaction mixture includes both the P_0† and the $\Delta 7P$† but not the P in glucose-6-phosphate, any conversion of $\Delta 7P$ to glucose-6-phosphate will be reflected in a decrease in the P_7 values during incubation. However, the calculation of hexokinase activity in complex systems by means of such changes poses a difficult problem since transphosphorylation from ATP may fail to account for all of the phosphate changes actually occurring.

In rat muscle preparations at least two other types of transfer, in addition to transphosphorylation, are often observed. Large increases in inorganic phosphate may occur, even in the presence of fluoride, due to the dephosphorylation of ATP by the apyrases[1] present in such preparations. In addition part of the inorganic phosphate formed during incubation may be derived from sources other than the $\Delta 7P$. The action of the apyrases is accompanied by a large increase in the P_0 content, a decrease in the $\Delta 7P$ and no change in the P_7 value. The increase in inorganic phosphate from extraneous reactions is manifested by increases in both the P_7 and P_0 values. The increase in the P_7 value indicates that fraction of the total increase in inorganic phosphate that is attributable to the dephosphorylation of esters other than those containing easily hydrolyzable phosphate.

In order to demonstrate hexokinase activity by means of phosphorous transfers, it is essential to compare the changes occurring during incubation in the absence and presence of glucose. If the addition of glucose failed to influence phosphate changes other than those occurring as the result of the transphosphorylation catalyzed by hexokinase, calculation of the activity from a determination of the effect of glucose on the easily hydrolyzable phosphate content would be entirely feasible.[2] There is good evidence, however, that the presence of glucose may influence other reactions. Meyerhof and Geliazkowa[3] demon-

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† The following symbols are used: P_0—inorganic phosphate determined prior to hydrolysis; P_7—inorganic phosphate content determined after 7 minutes' hydrolysis at 100°C in 1 N HCl; $\Delta 7P$—that fraction of esterified phosphate converted to inorganic phosphate by 7 minutes' hydrolysis at 100°C in 1 N HCl (*i. e.*, $P_7 - P_0$).

1 Meyerhof, O., *J. Biol. Chem.*, 1945, **157**, 105.

2 Colowick, S. P., and Price, W. H., *J. Biol. Chem.*, 1945, **157**, 415.

3 Meyerhof, O., and Geliazkowa, N., *Arch. Biochem.*, 1947, **12**, 405.