TABLE II. Effect of Salts Solution and 1.75×10^{-4} M Metrazol Addition upon Membrane Potential and Locomotion in Single *Amoeba proteus*.

	Condition	Membrane potential	Rate of locomotion
Cell #1	Normal	−42 mV	1960 μ^3/sec
	Control	−41	2010
	Metrazol	−23	3100
Cell #2	Normal	−34 mV	1010 μ^3/sec
	Control	−32	1090
	Metrazol	−24	1890

fect a great change in cell behavior, it must be concluded that the addition of Metrazol was the cause of altered membrane potential and locomotion.

The mechanism of Metrazol has not been established, although membrane involvement in potassium transport has been indicated(6, 7). Protozoan membrane potentials are reduced by increased extracellular potassium(2, 3,4,5). The reduced potential is probably related to altered concentration gradient effects as seen in other cells. It may be that in altering membrane permeability to potassium the Metrazol has caused the observed change in the membrane potential. However, this is speculation beyond the studies described and would require particular testing.

It is interesting to relate our findings to those of Bingley and Thompson(5), who report a lower membrane potential (less negativity) at the forward-moving pseudopod than at the cell's posterior region. We have not confirmed their results, but do find that the decreased membrane potential is accompanied by an increase in pseudopodal extension. The mechanism of Metrazol's action upon amoeba is not clear, but it does not seem related to generally accepted attitudes of membrane potential maintenance or alteration.

Summary. While under constant observation, the membrane potential and rate of locomotion of *Amoeba proteus* have been determined under normal conditions and in the presence of 1.75×10^{-4} M Metrazol. The membrane potential was changed from a normal average of −40.7 to −29.1 mV, and the rate of pseudopodal extension increased from a normal average of 1820 to 4080 μ^3 per second. The possible significance of these changes are considered in relation to other reports upon Metrazol action and protozoan behavior.

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Excretion of C^{14}-Bilirubin in Newborn Guinea Pigs.* (28937)

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Reduced glucuronide formation in neonatal liver has been well documented(1,2,3), but there is no direct information as to what extent this interferes with bilirubin excretion in bile. Moreover, recent studies(4) have suggested that in addition to impaired glucuronidation, fetal liver also exhibits a reduced excretory function. In the present investigation the capacity of newborn liver to conjugate and excrete near-physiologic loads of bile pigments was assayed with C^{14}-bilirubin.

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Materials and methods. Labeled unconjugated and conjugated bilirubin were prepared and dissolved for injection as described previously(4). Ten newborn (1-day-old) and 8 adult guinea pigs were studied.

In 3 newborn and all adult animals, the cystic duct was ligated, and the common bile duct cannulated, permitting drainage of the bile to the outside. Labeled pigments were then injected into the inferior vena cava and for the next 2 hours, bile was collected in 15 minute aliquots for radioassay(4). In 7 newborn guinea pigs, labeled pigment was injected without cannulation of the bile duct and the animals were permitted to recover from the light ether anesthesia. Two hours after the pigment administration, the guinea pigs were sacrificed and the amount of isotope excreted in the bile was estimated from the combined radioactivity in the gall bladder and gastrointestinal tract(4). At death, serum was collected for radioassay and in the newborn animals, radioactivity in brain, liver, kidneys and bladder urine was determined (4). Dehydration and hypoglycemia were prevented by subcutaneous injection of 1.5 ml of a 5% glucose solution. Plasma volume was estimated in 6 other newborn guinea pigs, using 4 mg of human albumin-I^{131} in 0.4 ml saline(5).

Results. As shown in Table I, in the 6 newborn guinea pigs injected with unconjugated bilirubin, average biliary excretion of the administered label was 38.1%, which was less than half of the amount excreted by the adult animals ($p<.002$)(6). In the 4 newborn pigs, injected with conjugated pigment, an average of 57.2% of the isotope appeared in the bile, which was approximately two-thirds of the amount of label excreted by the adult animals ($p<0.03$)(6). In all newborns, a larger fraction of the administered pigment was eliminated in the bile after injection of conjugated as compared with unconjugated bilirubin ($p<0.005$)(6). For both types of pigment, the maximal hepatic excretory rate was significantly lower than that in adult animals(Fig. 1).

The mean plasma volume of 6 newborn guinea pigs was 5.7% of body weight (range 4.0 to 7.3%). Based on this figure and the reported plasma volume in adult guinea pigs (7), the following values were calculated for total retention of label in the circulation 2 hours after intravenous administration of unconjugated C^{14}-bilirubin: newborn animals, 5.9% (range 1.6 to 15.1%); adult animals, 0.5% (range 0 to 1.0%) ($p<0.04$)(6). Following injection of conjugated C^{14}-bilirubin, retention of the label in the circulation of adult guinea pigs was 1.5% (range 1.0 to

TABLE I. Cumulative Excretion of Radioisotope in Bile of Newborn and Adult Guinea Pigs 2 Hours After Intravenous Injection of C^{14}-Labeled Bile Pigments.

Guinea pig		Pigment administered		Radioactivity in bile, % of administered dose
Age, day	Wt, g	Type	Load, μg/g	
1	83	unconjugated	.7	31.1
1	78	"	.8	42.6
1	117	"	1.4	42.8*
1	88	"	1.6	20.9
1	119	"	1.6	47.2
1	118	"	1.6	44.0
Mean		"	1.3	38.1
Adults (5)	526-1000	"	.3-1.5 (mean .9)	84-100* (mean 89.1)
1	126	conjugated	.4	50.6*
1	137	"	.7	53.6*
1	101	"	1.1	57.3
1	74	"	1.5	67.1
Mean		"	.9	57.2
Adults (3)	311-759	"	.1-1.9 (mean 1.0)	80-93* (mean 88.4)

* Bile collected by external biliary fistula.

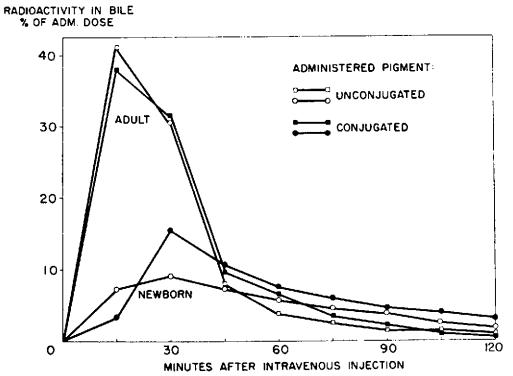

FIG. 1. Comparative rates of biliary isotope excretion after intravenous administration of unconjugated or conjugated C^{14}-bilirubin to newborn (1-day-old) and adult guinea pigs. Each curve represents one animal. Pigment loads were 1.4 and 0.7 μg/g (newborns) and 1.1 and 1.9 μg/g (adults).

2.4%) and of the newborn animals 6.2% (range 1.9 to 14.1%), a difference not statistically significant for this small series of experiments. In the newborn animals injected with unconjugated pigment, 28.8% of the label was present in the liver (range 8.7 to 44.8%), while only traces of isotope were detected in kidneys and urine, and none in the brain. After administration of conjugated bilirubin, 5.9% (range 5.7 to 6.1%) of the label was recovered in the liver, and traces were recovered in the urine.

Discussion. In newborn guinea pigs, the finding of reduced plasma disappearance and biliary excretion of injected unconjugated C^{14}-bilirubin supports previous reports that *in vitro,* glucuronide formation in neonatal liver is diminished(1). It is evident, however, that in relation to adult animals, the newborns' capacity to handle the pigment is less severely impaired than would have been expected on the basis of the greatly reduced glucuronide formation *in vitro* as assayed with o-aminophenol as substrate(1). In addition to this defect in conjugation, the liver of newborn guinea pigs was found to exhibit a diminished excretory capacity for conjugated C^{14}-bilirubin. A similar but more severe excretory defect had previously been observed in fetal animals(4). Nonetheless, as revealed by the data in Table I, in the one-day-old guinea pigs, comparable amounts of injected conjugated bilirubin were eliminated more efficiently than unconjugated pigment. This suggests that at this stage of development, conjugation and not excretion is the rate-limiting step in the overall handling of bile pigment.

Summary. Biliary excretion of injected unconjugated and conjugated C^{14}-bilirubin was compared in one-day-old and in adult guinea pigs. In relation to adult animals, in the newborn elimination of unconjugated pigment was reduced by more than half and that of conjugated pigment, by one-third. This indicated that in addition to impaired conjugation, the liver of newborn animals exhibits an excretory defect, but the former represents the rate-limiting step in overall pigment metabolism.

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Isozymic Patterns in Organs of Mice Infected with LDH Agent.* (28938)

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Riley *et al*(3) first called attention to a transmissible agent which caused elevation of the plasma lactic dehydrogenase (LDH) level. This agent, which was associated with transplantable mouse tumors, was shown to be a coincidental contaminant of tumors and readily separable from them. Until recently, the presence of the agent could be detected only by a rise in plasma LDH and some other enzymes, noted later to be elevated concomitantly(5,6). This rise apparently persists for the life time of the animals(11). Except for splenomegaly, no pathologic changes have been reported in organs of infected animals(12). Riley has recently published some evidence that the agent produces a hemolytic anemia and that the source of the rise in

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