# Passage of Metabolites of Chloral Hydrate into Amniotic Fluid. (20675)

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The question of the origin of the amniotic fluid is still controversial. Gusserov(1,2) proved experimentally that fetal urine is excreted into the fluid, but according to Schroeder(3) the amniotic fluid is originally a transudate to which the fetal urine is added afterwards. Plentl and Hutchinson(4) estimated that in the human an hourly exchange of fluid amounting to 550 cc to and from the amniotic fluid takes place. The collective evidence suggests a possible change from a straight transudate at the time preceding fetal kidney function to a more or less exclusive renal origin in the later stage of fetal life. In our studies with chloral hydrate and its transfer from the maternal organism to the foetus, we found that the foetus excreted a metabolite of chloral hydrate in its urine and this was recovered in the amniotic fluid.

Butler(5) has shown that after chloral hydrate administration the blood contains, in addition to chloral hydrate, trichloroethanol and trichloroacetic acid. He also described a method of determination of these compounds in the blood, according to which chloral hydrate and trichloroacetic acid are directly determined colorimetrically by the red color developed by heating with pyridine and strong sodium hydroxide solution. Trichloroethanol is determined by the same method after oxidation of trichloroacetic acid with sulfuric acid and potassium dichromate. The urine of healthy animals contains after chloral hydrate administration only urochloralic acid as sole metabolite(6). This conjugation product of trichloroethanol with glucuronic acid cannot be determined directly, but after hydrolysis and oxidation with chromic acid mixture to form trichloroacetic acid, it can be determined as this compound by the method as described.

*Methods.* It is known that chloral hydrate readily passes the placenta and circulates in the foetus; however, no investigation has been extended to the amniotic fluid. Since human amniotic fluid is easily contaminated with blood, experiments were performed with rabbits. 1) Rabbit of 5160 g, in fourth week of gestation, received by stomach tube 200 mg chloral hydrate as a 10% solution. After 30 minutes the animal received a high dose of pentobarbital sodium intravenously, was immediately opened, and the amniotic fluid was withdrawn with a hypodermic syringe from the embryonic sacs. The clear liquid did not give the pyridine reaction, which excludes the presence of chloral hydrate or trichloroacetic acid. Extraction with heptane did not show the presence of trichloroethanol. After evaporation of 1 cc to dryness and oxidation with 2 cc chromate-sulfuric acid mixture, the reaction was positive, proving the presence of urochloralic acid. Colorimetric measurements indicated an equivalent to 4 $\mu$g chloral hydrate per cc. The analysis of the maternal blood showed per cc: chloral hydrate 10 $\mu$g; trichloroacetic acid 2.5 $\mu$g and trichloroethanol 2 $\mu$g. 2) Rabbit of 4430 g, gestation of 3 weeks, received 0.3 g chloral hydrate as 10% solution in oil rectally; after 1 hour 0.3 g in 10% aqueous solutions was given orally. Twenty-five minutes later the animal was killed. The amniotic fluid contained only urochloralic acid, 9.5 $\mu$g chloral hydrate equivalents per cc. The blood of this animal contained per cc: chloral hydrate 48 $\mu$g; trichloroacetic acid 12.5 $\mu$g and trichloroethanol 3.5 $\mu$g. 3) Rabbits, 5 kg weight, on 12th day of gestation. This is the earliest time at which pregnancy can safely be determined. The animal received 300 mg chloral hydrate as 10% solution in oil rectally, after one hour 100 mg as 10% aqueous solution orally, and was killed one-half hour later.

From 14 foetuses two specimens of pooled fluid were collected each from 7 foetuses. A blood sample was drawn before the killing and a urine sample was taken from the filled bladder. The amniotic fluid was obtained in small quantity and was of viscous consistency. Since the high protein content interfered with

the color reaction, the liquid was treated with Folin's tungstic acid reagent and the tests carried out on the filtrate.

The analytical *results* were:

| | Sample I | Sample II |
|---|---|---|
| Chloral hydrate | 60 μg/cc | 50 μg/cc |
| Trichloroacetic acid | 22.5 | 15 |
| Urochloralic acid (calculated as chloral hydrate) | 44 | 35 |

Urine: Chloral hydrate and trichloroacetic acid negative; urochloralic acid: 560 μg/cc.

Blood: Trichloroethanol, 25 μg; trichloroacetic acid, 32.5 μg; chloral hydrate, 32.5 μg/cc.

In view of the small quantities of amniotic fluid collectable in the third rabbit, the potential error in the figures given is considered to be large, and the results should be evaluated rather as qualitative than quantitative.

It seems that at the time of gestation of the third rabbit, kidney function has set in and the transudative portion, containing the compounds circulating in the blood, is already mixed with urochloralic acid of urinary origin. At a later stage the urinary liquid replaces the reabsorbed transudate leading to a more watery fluid containing urochloralic acid only.

*Summary.* The results indicate that in the early stage of pregnancy chloral hydrate and its metabolites pass from the blood into the amniotic fluid. However, urochloralic acid, which is the form by which chloral hydrate is excreted by the kidneys, was found also at a stage when the gestation had progressed to two-fifths. In later pregnancy only urochloralic acid was found, which indicates that in the later stages the amniotic fluid contains essentially the products of foetal renal excretion.

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Received October 20, 1953. P.S.E.B.M., 1953, v84.

## Blockade of Ovulation in the Hen with Adrenolytic and Parasympatholytic Drugs.* (20676)

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The involvement of the nervous system in the release of the ovulatory hormone (LH) has been demonstrated in several mammals. Electrical stimulation of the hypothalamus of the rabbit causes ovulation at levels that are ineffective when applied directly to the pituitary gland(1,2). Similarly other types of experiments on the rat have also indicated a neural component in ovulation(3). Recently Markee, Everett & Sawyer(4) have reviewed the data indicating the presence of a neural and neurohumoral link in the release of LH not only in the rabbit which ovulates following copulation but also in the rat, a non-spontaneous ovulator. The presence of a 24-hour rhythm in the neural link was indicated by the ability of progesterone to advance ovulation by 24 hours in the rat(5) and the ability of the barbiturates to delay ovulation by approximately 24 hours(6,7). Nembutal is also effective in preventing progesterone induced ovulation in rats(8).

The present study was undertaken to clarify the role of the nervous system in ovulation in the domestic hen. Like the rat, the hen is a cyclic, spontaneous ovulator. However, the hen differs from the rat in that the ovulatory

* Aided by a grant from the Purdue Research Foundation.

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