

duct is bound up near the liver. Further, about 3 cm from the duodenum, a soft rubber tube of appropriate thickness is inserted into the bile duct and fixed. The other end of the tube, provided with the sillon net to prevent blocking of the tube with the killed worms, is inserted into the gall bladder. So the flow of bile is in no way interfered with, and no flukes are lost after being killed by the substance under trial. Using this method new anthelmintics have been examined on 75 sheep and the work continues.

The operation is performed in 'Thiopental^R' anaesthesia and usually lasts for about 35–45 min. Some hours later the anthelmintic is given. The animals are killed on the fourth or fifth day. By thorough helminthological dissection of livers the mature flukes are picked up and ascertained to what extent they became necrotic. In this way a direct comparison is made, and the percentage of worms killed or damaged is assessed exactly. By this method the action against immature stages of liver flukes could be examined, too. Apart from this the method is suitable for testing substances against *Dicrocoelium lanceolatum*.

Most of the animals lived after the operation as needed, but in the early stages of the work some animals succumbed before the fourth day. Some of the animals which were not killed lived for 3–6 months and one is still alive after more than a year.

The examination of some known anthelmintics (1,4-bis-trichloromethylbenzene, hexachlorophene, 'Freon 112') has shown that the method works well and enables one to perform critical test with substances acting against *Fasciola hepatica* in sheep.

JOZEF VODRÁŽKA

Department of Pharmacology,
Veterinary Faculty,
Agricultural College,
Košice, Czechoslovakia.

- ¹ Vodrážka, J., *Vet. Rec.*, **72**, 404 (1960).
² Enigk, K., and Düwel, D., *Deutsch. tierärztl. Wschr.*, **68**, 601 (1961).
³ Lämmler, G., *Arzneim.-Forsch.*, **5**, 497 (1955).
⁴ Lämmler, G., *Z. Trop. med. u. Paras.*, **7**, 289 (1956).
⁵ Lämmler, G., *Z. Trop. med. u. Paras.*, **10**, 379 (1959).
⁶ Plotnikov, I. N., *Raboty po gel'mintologii* (Izdat. Akad. nauk. Moskva, 1953).
⁷ Plotnikov, I. N., *Voprosy kraevoj patologii* (Medgiz. Moskva, 1957).

Resuscitation of Sheep Embryos and the Simulation of Intra-uterine Life

PRELIMINARY investigations made on the short-term revival of embryos obtained from sheep at slaughter suggest that extra-uterine maintenance of mammalian embryos is a technique awaiting development and exploitation.

Embryonic sheep between 5 and 33 cm CR-length (43–113 days¹; gestation period, approximately 150 days) were connected to a simply-constructed heart-lung machine with the roller-pump in series with the embryonic heart. An artificial amnion made of 'Perspex' was filled with 0.9 per cent sodium chloride at 39° C and through the wall of this tank entered two rubber-tubes which served as extensions for the umbilical vessels. Each tube was convoluted within the saline and then passed through the liquid surface to the operating table. The cannulae used for connecting these tubes to a foetus were transparent polyvinyl chloride or polythene ('Portex'). The output tubing from the pump passed to the tank and connected with the umbilical vein extension; the umbilical artery extension was taken from the tank to an oxygenator of the rotary-disk type^{2,3}. Heparinized Ringer solution and acid-citrate dextrose (ACD) were used as blood substitutes. Oxygen was metered into the disk-chamber in a direction opposing the flow of the liquid; after passing the disks the liquid was recirculated by the pump.

Before an experiment the oxygenator was charged with either solution and the cannulae mutually connected with plastic tubing. The pump was switched on and the liquid drawn from the oxygenator was passed down the umbilical vein extension through the joined cannulae to the umbilical artery-extension and returned to the oxygenator. The pump was stopped and the cannulae were disconnected at the operating-table level. Since this table was lower than the oxygenator there was now gravity-feed from the artery extension and this temporarily reversed flow was necessary for the insertion of that cannula. This flow was controlled by a clip and the flow from the vein extension by switching the pump.

Pregnant uteruses from the Bristol Public Abattoir were sealed in polythene bags and plunged into water at about 39° C. They were brought to the laboratory as quickly as possible, but the interval between killing the ewe and the dissection of its uterus was never less than 18 min.

A large cut was made through the uterine wall and foetal membrane of a conceptus, the incision running between and parallel to lines of cotyledons. As the foetus was lifted from the amniotic fluid and placed on sponge-rubber its cord was unravelled and gently stretched. An umbilical vein was entered after placing a linen-thread ready to ligate it; the pump was switched on and the stream of liquid from the cannula used to wash the site at which the vein was opened. When the vein was cannulated the pump was stopped until an artery was similarly connected, then the remaining umbilical vessels were clamped and the cord sectioned on the placental side of the connexions. The foetus was raised to just above the level of the meniscus in the oxygenator and the heart-pump switched on. If the pumping and connexions were satisfactory, the foetus, accompanied by cannulae and vessel extensions, was lowered into the amnioid cavity.

Six mechanically successful transfers were effected; 3 using ACD and 3 using heparinized Ringer. In all cases serious oedema developed within 15–20 min and the embryos, still connected, were taken from the saline for examination. In those receiving ACD and in one receiving Ringer no activity was observed on exposing the heart, but in the remaining two Ringer cases strong rhythmic activity was present with ventricular as well as atrial contraction. Since, apart from several temperature measurements, the biophysical system was unmonitored and the technique empirical it was expected that unphysiological conditions would be rapidly established. Neither perfusion fluid used could be expected to sustain the embryos for long and the use of whole blood should give better results.

I thank Miss J. Honour, who helped to construct the plastic parts of the apparatus, and Mr. T. Baker, who collected the biological material.

A. W. MARRABLE

Department of Veterinary Anatomy,
School of Veterinary Science,
University of Bristol.

- ¹ Joubert, D. M., *J. Agric. Res.*, **47**, 332 (1956).
² Garrott Allen, J., *Extracorporeal Circulation* (Charles C. Thomas, Springfield, 1958).
³ Bjork, V. O., *Lancet*, **255**, 491 (1948).

SOIL SCIENCE

Occurrence of a Previously Unobserved Nitrogen Gas in the Reaction Product of Nitrous Acid and Lignin

DURING investigations into the nature of gases produced by the reaction of nitrous acid with soil humic acids and lignins, a product was obtained from lignin which showed anomalous behaviour. There was a slow uptake of gas by permanganate solution beyond the time required for