Macrophages from guinea-pigs inoculated with human red cells provoked adherence, in vitro, of human red cells, irrespective of blood group or Rhesus factor. These macrophages adsorbed sheep and rabbit red cells on their surface to a lesser degree than macrophages of guinea-pigs inoculated with sheep red cells adsorbed human or rabbit erythrocytes. Controls performed with macrophages from normal guinea-pigs were negative. Macrophages from guinea-pigs inoculated with human sera (A, B, O) or pools of human sera, when sensitized in vitro with A or B serum, gave weak agglutination with the respective cells, according to the groups of the adsorbed sera and of the red cells (Figure 1b).

The groups of the inoculated sera had no bearing on the reaction. When sensitized in vitro with O serum, these macrophages agglutinate human red cells (A, B, O). This adherence was sometimes followed by phagocytosis of the erythrocytes attached on the surface of the macrophages.

The same macrophages provoked adherence of human red cells (A, B, O), without previous in vitro sensitization with any human sera.

Macrophages from non-inoculated guinea-pigs, brought into contact, in vitro, with human sera (A, B, O), did not provoke adherence of the corresponding human red cells, nor were they directly able to provoke adherence of human erythrocytes.

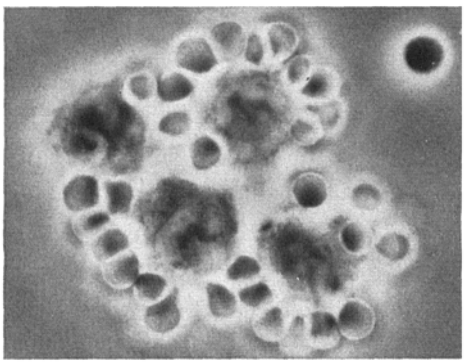

Fig. 2. Lysis of sheep red cells, when complement is added to the 'rosettes'.

On addition of complement (pool of fresh guinea-pig sera, absorbed with sheep red cells and diluted $^1/_{20}$) to 'rosettes' formed by macrophages of guinea-pigs inoculated with sheep red cells and by these red cells, lysis of the red cells attached to the macrophages occurred and red cell ghosts were observed around the macrophages (Figure 2). When lysed red cells were numerous, several macrophages seemed to be affected and to undergo lysis and disruption. Controls performed with unsuitable sera or red cells, or with normal or non-sensitized macrophages, were always negative.

Our experiments suggest that the macrophage reaction is species- but not group-specific. Weak cross reactions for sheep, human and rabbit red cells are attributable to the presence of a heterophile antigen other than FORSSMAN.

Experiments on the relationship of cytophilic antibody to other antibodies in different serum fractions are now in progress in our laboratory[9].

Résumé. Les macrophages de cobayes immunisés provoquent in vitro l'adhérence des hématies utilisées comme antigènes, selon une spécificité d'éspèce et non de groupe. Les sérums humains s'adsorbent in vitro sur les macrophages de cobayes immunisés par ces sérums, indépendemment du groupe (mais non sur des macrophages de cobayes normaux) causant l'adhérence et la phagocytose des hématies correspondantes. Les hématies adhérant aux macrophages sont hémolysées en présence du complément.

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Carcinogenesis in Different Animal Species by Diethylnitrosamine

Only a few carcinogenic compounds have been tested comparatively in different animal species. Mostly the experiments were carried out in rats and mice only and seldom in rabbits or dogs. Therefore many people are doubtful about the possibility of relating the results of experimental work to human beings, saying that the conditions for instance in rodents are quite different from those in men. To verify these objections we have tested diethylnitrosamine (DENA), whose carcinogenic actions were first observed by us in rats[1,2], also in mice[3,4], guinea-pigs[3], rabbits[5], dogs[6], monkeys[7], grass parakeets[8] and pigs[9]. The question was not only to check DENA in these animals but also to investigate the organotropy of the action and doses necessary to provoke cancer in all these animals. For that reason the experiments were done quantitatively.

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The carcinogenic action of diethylnitrosamine in different animal species

Animal species	Mode of application	Daily dose mg/kg	Total dose (D_{50}) mg/kg	Type of tumour	Authors
Mouse	orally	3	871 ± 124	Hämangioendotheliomas of the liver	4
Rat	orally	3	700 ± 53	Hepatocarcinomas	2
Hamster	orally	40 (weekly)	640	Hepatocarcinomas	15
Guinea-pig	orally	3	1200 ± 100	Hepatocarcinomas	3
Rabbit	orally	3.4	2500	Hepatocarcinomas	5
Dog	orally and s.c.	3	560	Leimyosarcoma of the liver	6
Pig	orally	4.4	1400	Reliculosarcomas of the liver	9
Monkey	orally	2–50	1400–25,700	Hepatocarcinomas	12
Grass parakeet	i.m.	70 (weekly)	2800 ± 400	Hepatocarcinomas	8
Brachydanio rerio	orally	10–100 ppm	10–13 weeks	Hepatocarcinomas and Cholangiomas	13
Trout				Hepatocarcinomas	14

Except for the investigation in grass parakeets (i.m. injection into the M. pectoralis once weekly, single dose 70 mg/kg), DENA was given in the other experiments orally in the drinking water in a daily dose of about 3 mg/kg body weight. Food consisted of Altromin-pellets ad libitum. All animals were observed until natural death, and then dissected and the important organs examined histologically[10]. The total doses needed for cancer production were a measure of the carcinogenic activity of the substance in the different animals. Dose-response curves were calculated according to a method already published[11].

Our results are compiled in the Table, which includes the results from other investigators on the same subject. Tumours of the liver arose in all animal species investigated. The frequency of the liver tumours was practically 100%. Also in guinea-pigs the carcinogenesis was complete. This was rather surprising because these animals were considered to be highly resistant to carcinogenesis by chemical substances. Only in monkeys did we fail to observe liver tumours, whilst KELLY et al.[12] produced liver cancer in these animals too, after feeding DENA. We observed severe liver damage in the monkeys with destruction of the architecture, necrosis of the organ, bleeding and proliferation of the bile ducts. Probably the life-time of our monkeys was too short for the development of cancer. Whilst we obtained hepatocarcinomas and occasional carcinomas of the oesophagus in rats, hepatocarcinomas in guinea-pigs, rabbits and grass parakeets, hemangioendotheliomas of the liver in mice and leiomysarcomas of the liver in dogs and reticulosarcomas of the liver in pigs, STANTON[13] and HALVER[14] saw hepatocarcinomas in fishes and HERROLD and DUNHAM[15] in the syrian golden hamster. Besides this, in the case of the hamster DENA produces also papillomas and carcinomas of the trachea and bronchus[15,16].

The total doses necessary for liver cancer production were in the same order of magnitude in all animal species tested (Table). In a probit net the dose action curves were highly characteristic and straight lines with a tg between 30 and 80. This is a question of normal distribution[4,17].

In some animal species (rabbits, dogs and pigs) treated with the daily dose of ~ 3 mg/kg DENA, liver cirrhosis developed besides liver cancer, whilst in other animals (mice, rats, parakeets, guinea-pigs) no cirrhosis arose but only liver cancer. We assume the larger the animals the better the conditions for cirrhogenic action. However, both actions (carcinogenic and cirrhogenic) are not necessarily connected.

Our investigations and those of others show that DENA produces cancer of the liver in all 11 animal species tested up to now. All animal species react rather equally. Therefore in men too the hepatocarcinogenic action of DENA is highly probable.

Zusammenfassung. Die hepato-cancerogene Wirkung von Diäthylnitrosamin konnte an 11 verschiedenen Tierarten in chronisch-toxikologischen Untersuchungen nachgewiesen werden. Die zur Krebserzeugung benötigten Gesamtdosen liegen bei allen Tierarten in der gleichen Grössenordnung. Auch beim Menschen ist eine leberkrebserzeugende Wirkung von Diäthylnitrosamin sehr wahrscheinlich.

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