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## THE CARCINOGENIC EFFECTS OF 6-AMINOCHRYSENE ADMINISTERED TO NEWBORN MICE

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In 1937, Haddow and Robinson showed that chrysene – a polycyclic hydrocarbon which is weakly carcinogenic – decreased the rate of growth of the Jensen sarcoma and the Walker carcinosarcoma in rats. Some years later, Lacassagne *et al.* (1945) reported that chrysene produced 'weak but definite' inhibition of the carcinogenic activity of 20-methylcholanthrene, measured by the induction of epithelial skin tumours in mice. These results suggested that modification of the chemical structure of chrysene might boost its inhibitory activity and reduce or eliminate its weak carcinogenicity; and in 1953, Rudali *et al.* described the properties of an amino derivative, *6-aminochrysene* (Fig. 1).



Fig. 1. Structural formula of 6-aminochrysene.

The growth-inhibitory effects of this compound were demonstrated most convincingly against skin tumours induced with 20-methylcholanthrene (MeC) in mice. In animals also given 6-aminochrysene, fewer skin tumours were produced, their latent period was longer and a smaller proportion underwent malignant change. The effects of 6-aminochrysene on spontaneous mammary tumours were less obvious: overall survival was not affected but the tumours grew more slowly in the treated animals and there seemed to be an increased incidence of regressions. Huggins *et al.* (1964) later showed that 6-aminochrysene, together with certain other polycyclic hydrocarbons, inhibited the development of mammary tumours in young female Sprague-Dawley rats, treated with one massive dose of 7,12-dimethylbenz[ $\alpha$ ]anthracene (DMBA). The overall incidence of mammary tumours fell in rats given 6-aminochrysene, the number of tumours developing in each animal was reduced, and their latent period rose. 6-Aminochrysene has now been tested in a small number of patients with advanced carcinoma of the breast, and objective regression observed in 2 out of 32 cases (Groupe Européen du Cancer du Sein, 1967).

In addition to the tumour-inhibitory properties of 6-aminochrysene, Rudali *et al.* (1953) noted that this substance produced splenic atrophy in rats and mice. Further work by Rudali and Buu-Hoï (1955) showed that splenic atrophy was accompanied by leucopenia in the peripheral blood, mainly due to a fall in circulating lymphocytes. These effects have also been exploited in clinical medicine, particularly in the treatment of tropical and other splenomegalies (Buu-Hoï *et al.*, 1962; Payet *et al.*, 1963). 6-Aminochrysene has

been ineffective in lymphocytic leukaemia but it has been used in the treatment of myeloid leukaemia (Hugonot, 1964).

The clinical use of 6-aminochrysene has been facilitated by its unusually low toxicity (Rudali *et al.*, 1953; Hugonot, 1964; Lambelin *et al.*, 1967) and by an apparent lack of carcinogenic activity. Tests for carcinogenesis have been consistently negative in adult rats and mice, irrespective of the route of administration of 6-aminochrysene whether by application to the skin, subcutaneous or intramuscular injection, or incorporation in the food and this inactivity has been stressed by several writers (Rudali *et al.*, 1953; Hugonot, 1964; Lambelin *et al.*, 1967). The carcinogenic effects of 6-aminochrysene in newborn (as opposed to adult) animals have not, however, been reported and there is now evidence that this substance induces many hepatic and pulmonary tumours when injected into mice during the first 3 days of life (Roe *et al.*, 1969).

In the present experiment, 85 newborn Swiss albino mice -43 males and 42 females – were divided into test and control groups. Animals in the test group -23 males and 23 females – received subcutaneous injections of 200 µg 6-aminochrysene/0.02 ml arachis oil on the first, second and third days of life. Animals in the control groups -20 males and 19 females – received three subcutaneous injections of 0.02 ml arachis oil only, at the same times. Details of treatment are summarised in Table I. All mice were maintained

### TABLE I

	Summary of main experimental details				
TEST G	ROUP				
<b>*</b> 9	23 23	{	200 $\mu$ g 6-aminochrysene/0.02 ml arachis oil Injected subcutaneously on first 3 days of life		
CONTRO	DL GRC	OUP			
<b>ð</b> 9	20 19	\$	0.02 ml arachis oil Injected subcutaneously on first 3 days of life		

in conventional conditions and, after weaning at 5 weeks, were housed in groups of 5 and fed on a standard cubed diet and water.

Between 9 and 11 months after the start of the experiment, 7 males from the test group died or had to be killed because of abdominal lumps. One female from the test group and 2 male mice from the control group also died during this time. The pathological findings in these 10 mice are shown in Table II: the main feature is the high incidence of hepatic and pulmonary tumours, confined to male mice injected neonatally with 6-aminochrysene.

after beginning of experiment					
	TUMOURS				
	Liver	Lung	Other sites		
TEST GROUP					
ð 7	7/7	5/7			
P 1					
CONTROL GROUP					
J 2					
♀ <b>0</b>					

	TABLE II	
Autopsy	findings in 10 mice dying 9-11 after beginning of experiment	months

168

#### CARCINOGENIC EFFECTS OF 6-AMINOCHRYSENE

In view of these findings at 11 months, it was decided to kill 5 male and 5 female mice from the test and control groups to determine the distribution of hepatic and pulmonary tumours in these 20 animals. Full post-mortem examinations were made and tissues with tumours were removed and fixed in Bouin's solution. Paraffin sections were prepared at 5  $\mu$  and stained with haematoxylin and eosin and by periodic acid-Schiff (PAS). The results, combined with the earlier findings, are shown in Table III. The high incidence of hepatic and pulmonary tumours in male test mice is confirmed; only 2 of the female

TABLE III						
Summary	of	autopsy	findings	in	30	mice

	TUMOURS			
	Liver	Lung	Other sites	
TEST GROUP				
₹ 12	12/12	9/12	0/12	
<b>ç</b> 6	1/6	1/6	0/6	
CONTROL GROUP				
ð 7	1/7	0/7	0/7	
₽́ <b>5</b>	0/5	0/5	0/5	

test mice developed hepatic or pulmonary neoplasms; and only 1 of the untreated control animals (a male) developed hepatic tumours. No other neoplasms were found in any mice from the test and control groups.

The *liver tumours*, all of which were hepatomas, varied in size from 0.1 to 3 cm in diameter. They were commonly multiple and the amount of normal liver tissue was



*Fig. 2.* Well-differentiated hepatoma from a male mouse, injected neonatally with 600  $\mu$ g 6-aminochrysene. Haematoxylin and eosin.  $\times$  160 (reduced 25%).

## R. L. CARTER AND F. J. C. ROE

sometimes strikingly reduced. Most of the lesions were well differentiated (Fig. 2) and usually arranged in solid cords. The predominant cells were small with slightly basophilic cytoplasm but several tumours showed fatty change which was sometimes intense; most of them contained little or no stainable glycogen. Nuclear or cytoplasmic inclusions were rarely seen. Three tumours were poorly differentiated and showed a more open reticular arrangement with islands of pleomorphic cells separated by irregular blood-filled spaces (Fig. 3). Despite the considerable distortion of normal structure, the hepatic capsule was



Fig. 3. More anaplastic hepatoma from a male mouse, injected neonatally with 600  $\mu$ g 6-aminochrysene. Haematoxylin and eosin.  $\times$  160 (reduced 25%).

always preserved intact and there was no evidence of extension of tumour outside the liver, or of distant metastases.

The *lung tumours* were also usually multiple and varied in size from tiny nodules, less than 0.1 cm in diameter, to large masses replacing all or part of one lobe. All but one of the tumours examined were typical papillary adenomas (Fig. 4). Some of the lesions, though well differentiated, showed extension into adjacent bronchioles. One tumour was an unequivocal adenocarcinoma in which the orderly papillary arrangement was largely obscured by sheets of more pleomorphic cells. No extrapulmonary metastases were found.

The hepatic and pulmonary tumours induced by neonatal injections of 6-aminochrysene showed no special pathological features and were indistinguishable from hepatomas or pulmonary adenomas induced in adult Swiss mice by various means or arising spontaneously.

These results are of interest for various reasons: they confirm the value of including newborn animals in tests for carcinogenicity, particularly for substances found negative in tests with adult animals; they provide another example of the curious sex difference in susceptibility to carcinogens administered during the neonatal period; and they illustrate the association between tumour-inhibitory and tumour-inducing activity – an association particularly evident with certain polycyclic hydrocarbons and related heterocyclic compounds.



*Fig. 4.* Papillary adenoma of the lung from a male mouse, injected neonatally with 600  $\mu$ g 6-aminochrysene. Haematoxylin and eosin.  $\times$  160 (reduced 25%).

Interest in the activity of chemical carcinogens in neonatal animals was initially stimulated by studies on viral oncogenesis, for example, with SV4O, polyoma and murine lymphoma viruses (Pietra *et al.*, 1959). As a broad generalisation, newborn animals are usually more susceptible than adults with respect to the induction of lymphomas, pulmonary adenomas and hepatomas by chemical carcinogens, and are less susceptible to induction of tumours of the breast, subcutaneous tissues and skin by these agents; but many factors are involved such as the dose of the compound and the species, strain and sex of the animal tested (Toth, 1968; Della Porta and Terracini, 1969). In the present experiment, the use of neonatal mice has demonstrated definite carcinogenicity in a substance hitherto regarded as inactive in all tests involving adult animals. It seems reasonable to suggest that compounds should not be dismissed as non-carcinogenic until they have been tested in newborn animals, as well as in adults (Gorrod *et al.*, 1968).

The difference in susceptibility of male and female mice, injected neonatally with certain carcinogens, is well known. It is best illustrated by the hepatic tumours found predominantly or exclusively in male mice injected with polycyclic hydrocarbons such as DMBA and MeC (Klein, 1959; Roe and Walters, 1967), with 4-aminobiphenyl and some of its hydroxylated derivatives (Gorrod *et al.*, 1968), with griseofulvin (Epstein *et al.*, 1967), or with maleic hydrazide (Epstein and Mantel, 1968). The findings with 6-aminochrysene provide another example of the proclivity of male mice to develop hepatomas, but they differ from previous reports in that there is also a high incidence of pulmonary tumours among these animals. The *simultaneous* induction of hepatomas and pulmonary adenomas at high incidence in newborn animals has not been observed previously with other polycyclic hydrocarbons though a variable increase in the incidence of coexistent hepatic and pulmonary tumours has been described after the neonatal administration of dimethylnitrosamine (Toth *et al.*, 1964), o-aminoazotoluene (Nishizuka *et al.*, 1965) and, particularly, urethane (Della Porta *et al.*, 1963; Liebelt *et al.*, 1964; Klein, 1966; Matsuyama and Suzuki, 1968). But these various findings differ from the present observations in that the pulmonary tumours occurred in males and females in comparable proportions; pulmonary adenomas in animals given 6-aminochrysene have developed almost entirely in males.

It is not known why mice injected neonatally with certain carcinogens are particularly prone to develop tumours such as hepatomas or pulmonary adenomas. Several factors have been considered such as metabolic immaturity of detoxifying and other chemical pathways, functional immaturity of the target organs, immunological immaturity and activation of latent oncogenic viruses (Della Porta and Terracini, 1969). There is good evidence that the catabolism of DMBA and urethane is inefficient in newborn animals (Domsky et al., 1963; Boiato et al., 1966; Sims and Grover, 1967) but the basis for the remarkable sex difference in susceptibility to hepatic carcinogens is completely obscure. A difference of this magnitude suggests different detoxifying metabolic pathways in young males and females but there is, as yet, no evidence to support such a view.

Lastly, the present findings are interesting in that they illustrate the paradoxical relationship between tumour-inducing and tumour-inhibitory activity noted by Haddow in 1935. A similar relationship is seen with X-rays and with alkylating agents but its biological significance is uncertain at the present time.

#### SUMMARY

6-Aminochrysene is a tumour-inhibitory agent which is not carcinogenic to adult rats and mice. If, however, it is administered to newborn animals, a high incidence of hepatic and pulmonary tumours is subsequently found among the males.

23 male and 23 female Swiss albino mice received subcutaneous injections of 200  $\mu$ g 6-aminochrysene/0.02 ml arachis oil on the first, second and third days of life (total dose 600 µg). A further 20 males and 19 females acted as control animals and received 3 subcutaneous injections of 0.02 ml arachis oil only. Between 9 and 11 months after the start of the experiment, 7 treated males died or had to be killed because of abdominal masses; one female from the test group and 2 males from the control group also died during this time. At autopsy, all 7 male test mice had hepatomas, and 5 of these 7 animals also had pulmonary tumours; no tumours were found in the other mice killed at this time.

5 male and 5 female mice from the test and control groups were then killed to determine the distribution of hepatic and pulmonary tumours. The incidence of hepatic and pulmonary tumours in the different groups was as follows:

Test group

(a) Males: hepatomas 12/12, pulmonary tumours 9/12; (b) Females: hepatomas 1/6, pulmonary tumours 1/6.

Control group

(a) Males: hepatomas 0/7, pulmonary tumours 0/7;

(b) Females: hepatomas 0/5, lung tumours 0/5.

These results confirm the value of ueonatal animals in tests for carcinogenicity; they provide another example of the particular susceptibility of males which have been injected neonatally with certain carcinogens; and they illustrate the common and paradoxical association between tumour-inhibiting and tumour-inducing activity.

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