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CARCINOGENICITY OF ANALGESICS: LONG-TERM TREATMENT OF SPRAGUE-DAWLEY RATS WITH PHENACETIN, PHENAZONE, CAFFEINE AND PARACETAMOL (ACETAMIDOPHEN)

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Six groups of male Sprague-Dawley rats were treated with phenacetin, phenazone or caffeine in the diet or with combinations of these chemicals. Another group received paracetamol in the diet and a further group received only the control diet. The rats were treated for up to 117 weeks. Renal pelvic tumors were only seen in rats treated with phenacetin or phenazone alone or in combination with caffeine, phenazone having slightly greater activity toward the urinary tract than phenacetin. Phenacetin, however, had a greater overall carcinogenic effect, inducing 31 malignant tumors. The urinary tract and the kidneys had the highest incidence of tumor followed by squamous-cell carcinomas of the head and neck. Half of the rats treated with phenacetin, phenazone and caffeine in combination developed hepatomas. The explanation may be that the addition of phenazone and caffeine altered the metabolism of phenacetin, increasing the production of *N*-hydroxyphenacetin, a known liver carcinogen. The justification of using phenacetin as a human analgesic must be seriously questioned, and further studies with phenazone are required.

The association in humans between a high consumption of analgesics containing phenacetin, phenazone and caffeine and the development of renal pelvic tumors is well established (Johansson *et al.*, 1974). Phenacetin is an aromatic amide with carcinogenic *N*-hydroxylated metabolites (Calder *et al.*, 1976). In a previous study, administration of 0.535% phenacetin in the diet to Sprague-Dawley rats for up to 110 weeks induced a high incidence of urothelial hyperplasia of the renal papillae but no renal pelvic tumors (Johansson and Angervall, 1976). In the same study, earduct tumors developed in four rats and mammary adenocarcinomas in five, compared with one adenocarcinoma in a control rat. This suggested a more general carcinogenic effect of phenacetin in rats. Recently Isaka and co-workers (1979) demonstrated a high incidence of nasal carcinomas and urinary tract tumors after long-term administration of 1.25% and 2.5% phenacetin in the diet.

Patients whose consumption of analgesics has become abusive have almost invariably been taking mixtures of analgesics. In Sweden, the majority have been taking compositions containing phenacetin, phenazone and caffeine. No experimental studies appear in the literature concerning the general effects of long-term administration of phenazone, caffeine or paracetamol (the main metabolite of phenacetin) alone or in combination. Therefore the present study was performed.

MATERIAL AND METHODS

Experimental design

Two-hundred-and-forty 6-week-old, weanling SPF male Sprague-Dawley rats (Anticimex AB, Stockholm, Sweden) were used. The initial weight was approximately 50 g. The rats were divided into groups of thirty, and were given the following doses of chemicals in the diet:

Group 1, 0.535% phenacetin; group 2, 0.535% phenazone; group 3, 0.102% caffeine; group 4, 0.535% phenacetin, 0.535% phenazone and 0.102% caffeine; group 5, 0.535% phenacetin and 0.535% phenazone; group 6, 0.535% phenacetin and 0.102% caffeine; group 7, 0.535% paracetamol (acetamidophen). Group 8, received a control diet with no added chemicals.

Phenacetin and phenazone were supplied by Hoechst AG, Frankfurt, FRG, caffeine by Boehringer & Sohn, Ingelheim, FRG, and paracetamol by Chemfabrik Aubing, München, FRG. The purity of the drugs was tested by Apoteksbolagets Centrallaboratorium, Stockholm. The purity of phenacetin was 99.2-99.6%. Phenacetin was used as crystals with a particle size varying between 5 and 200 μm. The purity of caffeine was 99.6-99.9%, of phenazone, 99.2-99.8%, and of paracetamol, 99.5-99.7%. The diets were specially prepared by Astra-Ewos AB every second or third month. The pellets were prepared in a hand machine and the temperature never exceeded 45°C according to the manufacturer. The food was stored in dry dark quarters at 19°C±1. The phenacetin-containing diets were tested for purity and even after being stored for 18 months the purity of phenacetin in the diets was between 99.2 and 99.5%. No *p*-phenetidin was detected in the crystals or in the pelleted food.

Three to five rats were kept in each makrolon cage covered with softwood bedding, and given food and tap water *ad libitum*. The rats were exposed to cycles of 12 h light and 12 h darkness and were kept at a constant temperature of 22°C and at a relative humidity of 55%. The rats were weighed every other week and killed when moribund or at the end of the experiment, after 117 weeks. Food consumption was determined by weighing the food per cage every week, and dividing the amount of consumed food by the number of rats in the cage. The total food consumption for each rat was added up and multiplied by the percentage of chemicals in the diets, which gave the amount of chemicals consumed in grams per rat.

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Morphological methods and statistics

The rats were killed by a lethal dose of phenobarbitol. The abdominal cavity was opened and the urinary bladder inflated with Bouin's solution. Complete post-mortem examination was performed and histological sections were taken from the urinary bladder, kidneys, adrenals, liver, stomach, spleen, lungs, and heart, as well as from other grossly abnormal tissue. The kidneys, liver and spleen were weighed. Five-μm and 1-μm sections were made from paraffin-embedded and metachrylate-embedded tissue, respectively, then stained with hematoxylin and eosin and according to Weigert–van Gieson. To determine differences of means, Student's t-test was used. Means are given with the standard error of the mean.

Renal pelvic tumors

As can be seen in Tables III and IV, 13 renal pelvic tumors were found. The following histological appearances and distribution were observed:

Group 1: one well-differentiated non-invasive urothelial tumor. Group 2: two urothelial tumors invading the kidney; two moderately well-differentiated squamous-cell carcinomas invading the kidney and hilar fat tissue. Group 4: two non-invasive urothelial well-differentiated tumors; one mixed urothelial and squamous-cell carcinoma invading the kidney and hilar fat tissue with pulmonary metastasis; one moderately well-differentiated squamous-cell carcinoma invading the kidney and hilar fat tissue. Group 5: one poorly differentiated urothelial tumor invading the kidney; one moderately well-differentiated non-invasive urothelial tumor; one moderately well-differentiated squamous-cell carcinoma invading the kidney and hilar fat tissue. Group 6: one moderately differentiated urothelial tumor invading the kidney. Groups 3, 7, 8: no renal pelvic tumors.

TABLE I

AVERAGE LIFE SPAN AND ANALGESIC CONSUMPTION OF RATS TREATED WITH TEST CHEMICALS

Group	Mean survival (weeks)	Range	Consumption of chemicals (g/rat)			
			Phenacetin	Phenazone	Caffeine	NAPA
1 (30)	101	63-117	84.9			
2 (30)	92	66-114		90.2		
3 (30)	78	41-107			21.4	
4 (30)	101	29-117	83.7	83.7	25.0	
5 (30)	104	66-117	91.7	91.7		
6 (30)	92	46-116	85.8		25.7	
7 (30)	93	65-116				86.5
Control (30)	94	67-116				

Number of rats in each group given in brackets.

RESULTS

The average life-span, dietary intake, and consumption of chemicals are shown in Table I. The average life-span was significantly shorter in the group fed caffeine (group 3). Dietary intake was highest among the control rats and lowest among the caffeine-fed rats. The mean body weights at 74 weeks are given in Table II. The mean body weight of the control rats (group 8) was significantly higher ($p<0.05$) than that of the rats in the other groups except for those fed paracetamol (group 7).

Table III shows the type and frequency of different tumors detected in the various groups.

Lesions of the kidney and the urinary tract.

Tumors of the renal parenchyma. Renal cell tumors were found in 15 rats. Four of them were detected in group 1, two in group 4, seven in group 5, and two in group 6. The tumors measured 0.5-1.5 cm and were of clear-cell type (Fig. 1), granular cell type, or oncocytic cell type. The tumors exhibited mild to moderate atypia and few mitoses. One rat had bilateral tumors. No metastases were detected. In one caffeine-treated rat, a 7 × 6 × 5 cm tumor was found, which was histologically consistent with nephroblastoma.

TABLE II

MEAN BODY WEIGHT AT 74 WEEKS IN CONTROL AND EXPERIMENTAL GROUPS[1]

Group	Mean body weight (g)
1 (27)[2]	538± 8
2 (28)	595± 9
3 (18)	530± 9
4 (28)	473± 9
5 (29)	547± 7
6 (27)	493± 8
7 (27)	643±10
8 (28) controls	640± 9

[1]Group 1 received phenacetin, group 2 phenazone, group 3 caffeine, group 4 phenacetin, phenazone and caffeine, group 5 phenacetin and phenazone, group 6 phenacetin and caffeine, group 7 paracetamol, group 8 control diet. – [2]Number of rats in each group given in brackets.

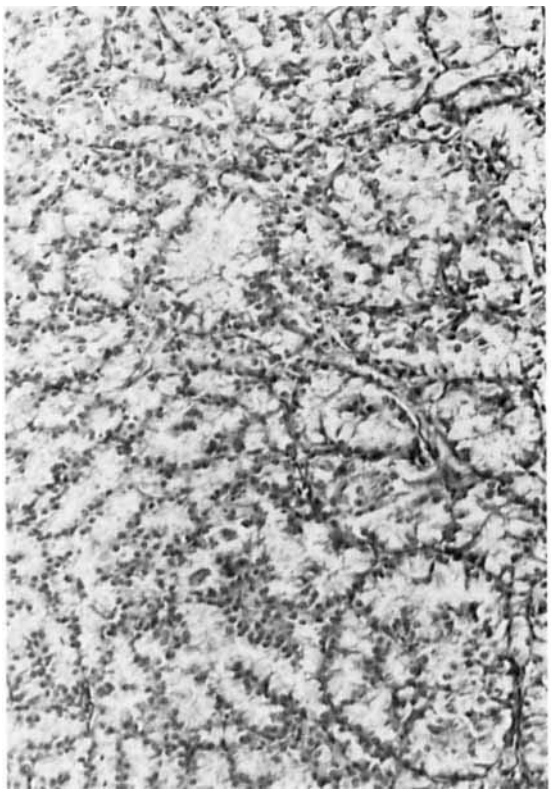

FIGURE 1 — Clear-cell tumor of the kidney parenchyma in a rat fed phenacetin and caffeine. H and E., × 120.

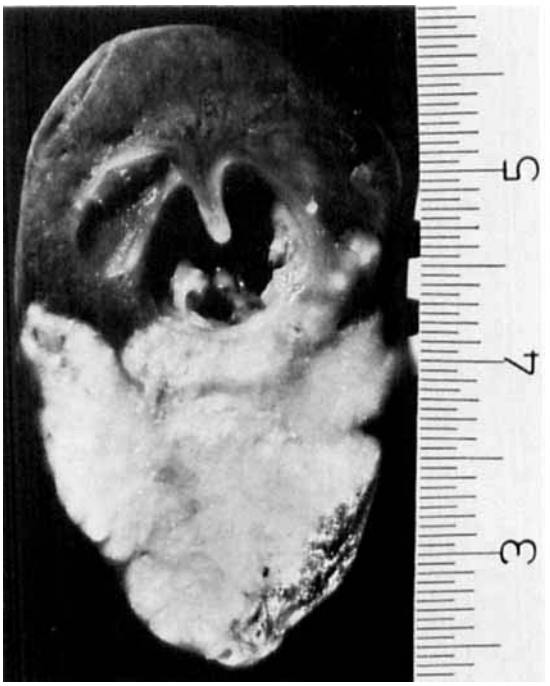

FIGURE 2 — Gross appearance of a squamous-cell carcinoma of the renal pelvis in a rat fed phenacetin, phenazone and caffeine for 84 weeks.

Thus, in summary, eight of the tumors were urothelial (transitional-cell tumors), four of which were invading the kidney, four were highly invasive squamous-cell carcinomas, and one was an invasive and metastasizing mixed urothelial and squamous-cell carcinoma (Figs. 2, 3, 4, 5, 6, 7).

Lesions of the urinary passages

The incidence of inflammatory and neoplastic lesions of the urinary passages are given in Table IV. Urinary bladder tumors or papillomatosis (Fig. 8) were found in five rats treated with phenacetin or

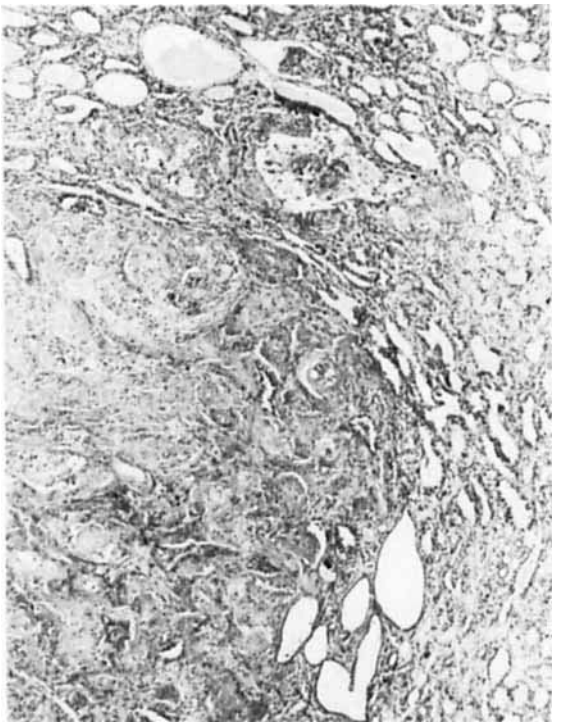

FIGURE 3 — Poorly-differentiated squamous-cell carcinoma invading the kidney. H. and E., × 48.

phenazone alone and four in each of groups, 5, 6, and 7. Finally, two control rats had similar lesions. The lesions were all urothelial, but small areas of squamous differentiation were seen in one rat in each of the groups treated with phenacetin, phenazone and paracetamol, respectively, and one in the control group. There was, however, a striking difference in the incidence of urinary tract infection between the different groups. Thus, 50% of the control rats had cystitis or prostatitis and 33% had pyelitis. Cystopyelitis was found in 33% of the paracetamol-treated rats. The rats in groups 1, 3, 4, and 6 had a significantly lower incidence of urinary tract infections. The incidence of urothelial hyperplasia of the renal pelvis is also tabulated in Table IV. The lesions were particularly frequent in the upper urinary tract among the rats treated with phenacetin

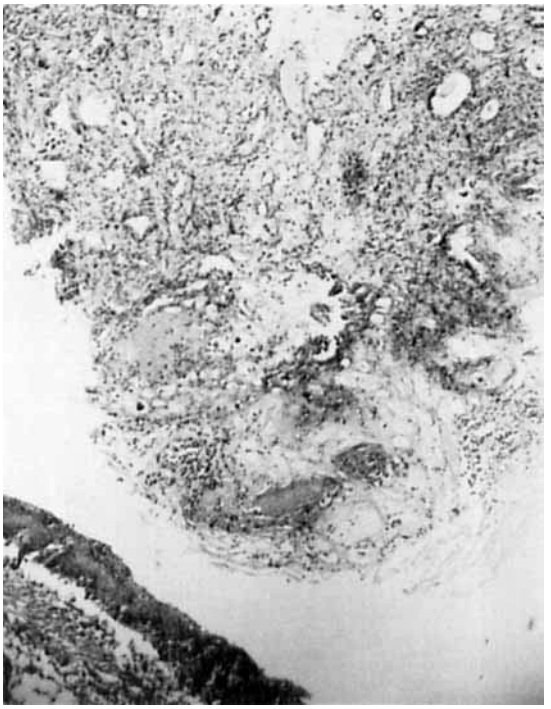

FIGURE 4 — Squamous-cell carcinoma growing in the renal papillae. Note squamous metaplasia on the opposite renal pelvis. Weigert–van Gieson, × 48.

only and were not associated with inflammation, in contrast to the lesions among the paracetamol-treated and control rats.

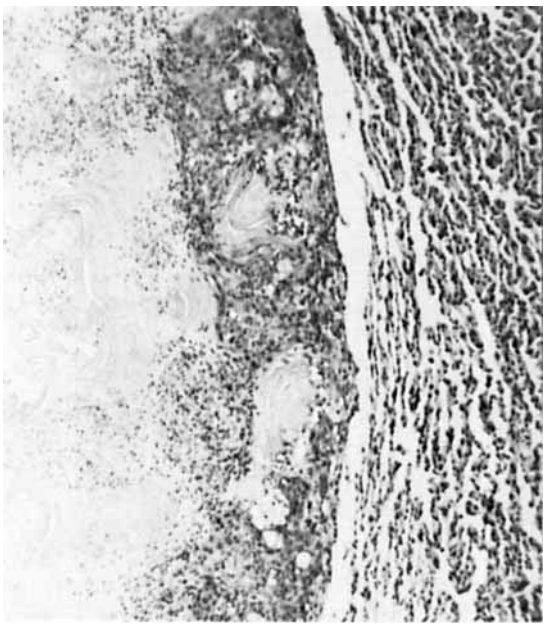

FIGURE 5 — Pulmonary metastasis of a keratinizing squamous-cell carcinoma of the renal pelvis. H. and E., × 120.

Renal papillary necrosis was found in one rat in group 1, five in group 4, three in groups 5 and 6, and two in group 7. The two latter lesions were associated with severe pyelonephritis.

Squamous-cell tumors

Squamous-cell carcinomas of various locations were the most common type of tumor. In the rats treated with phenacetin, seven of 31 malignant or potentially malignant tumors were found to be squamous-cell carcinomas. In this group, six of seven tumors appeared in the head and neck region (ear

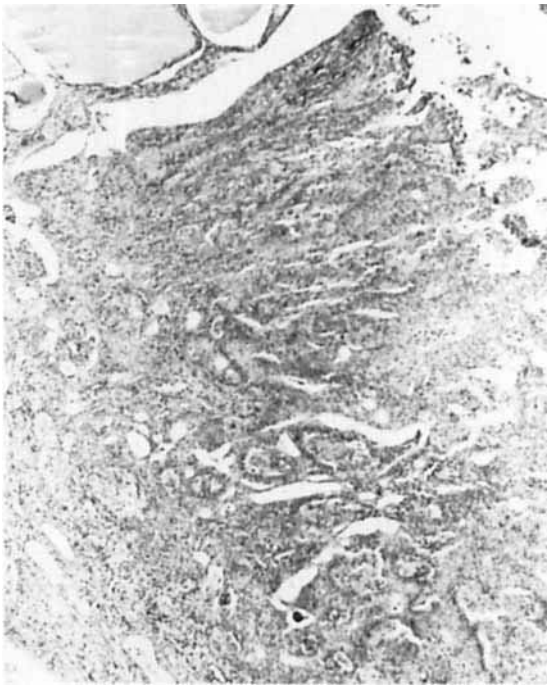

FIGURE 6 — Moderately differentiated, superficially invasive urothelial renal pelvic tumor in a rat treated with phenacetin and caffeine. Weigert-van Gieson, × 48.

duct, nose, oral cavity). Squamous-cell carcinomas in group 1 were detected after 90-109 weeks of feeding the chemical. One of the tumors localized in the oral cavity, histologically a moderately to poorly differentiated squamous-cell carcinoma, metastasized to the lungs. Squamous-cell carcinomas were also detected in all the other groups: three in group 3, two in each of groups 4, 5, 6, and 7, and one among the control rats (Table III). One moderately differentiated squamous-cell carcinoma originating in the bulbo-urethral glands in group 3 metastasized to the lungs. The earliest detected squamous-cell carcinoma was in the oral cavity in group 3 which appeared after 46 weeks of feeding the chemical. A squamous-cell carcinoma located in the oral cavity of a control rat was detected after 102 weeks.

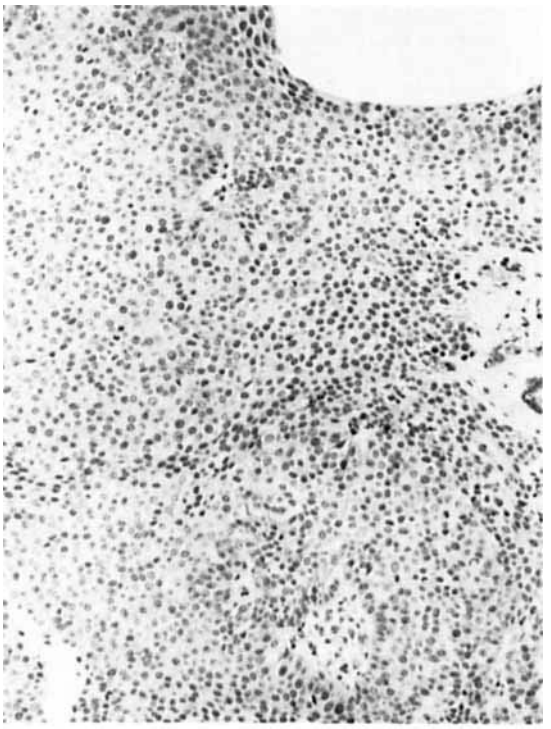

FIGURE 7 — Well-differentiated urothelial tumor in a rat treated with phenacetin only. H. and E., × 120.

In 11 rats, forestomach tumors were found, six of them among the rats treated with phenacetin and phenazone. Nine of the tumors were squamous-cell papillomas. Two tumors (in groups 5 and 6) were invasive and thus classified as well-differentiated squamous-cell carcinomas.

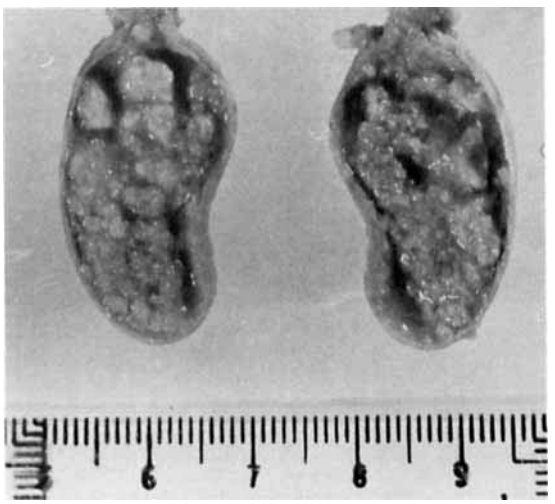

FIGURE 8 — Papillomatosis of the urinary bladder in a phenacetin-treated rat.

Tumors of the liver

Fourteen of the 30 rats treated with the combination of phenacetin, phenazone and caffeine were found to have hepatoma. The tumors were detected at autopsy at 104-116 weeks. The tumors were multiple, measuring between 0.5-2.0 cm. Histologically the tumors were moderately well-differentiated hepatocellular carcinomas (Fig. 9). Focal nodular hyperplasia was seen in these rats as well as in five other rats in group 4 without hepatomas. Focal nodular hyperplasia was also seen in 12 rats in group 5, and eight in group 6, but no tumors were detected in these groups.

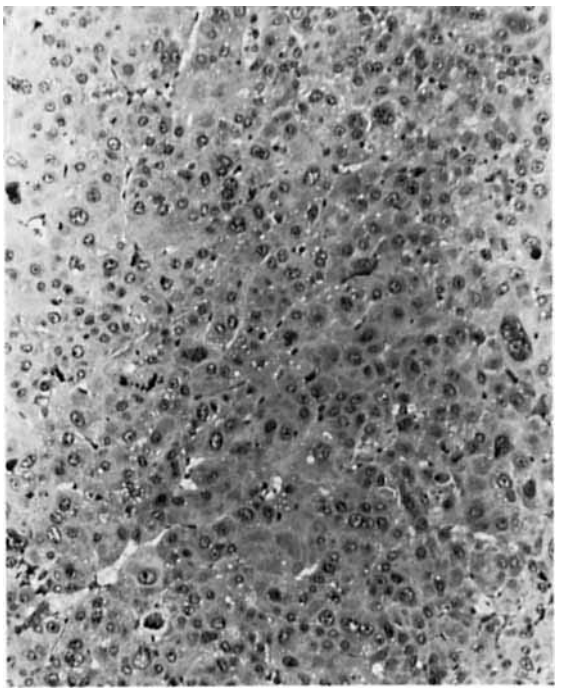

FIGURE 9 — Histology of the liver showing moderately well-differentiated hepatoma. H. and E., × 120.

Two cystic cholangiomas were found after 75 and 114 weeks respectively among the phenacetin-treated rats, and one similar tumor after 115 weeks among the rats treated with the combination of phenacetin and phenazone. One cavernous hemangioma was detected after 115 weeks among the rats treated with phenacetin only.

Adenocarcinomas

Only six adenocarcinomas were found, three of them among the phenacetin-fed rats (Table III). The lung tumor found after 88 weeks of phenacetin-treatment had metastasized to the regional lymph nodes, and in the same group a carcinoma of the common bile duct with pulmonary metastases was found after 115 weeks.

TABLE III

THE TYPE, DISTRIBUTION AND FREQUENCY OF TUMORS IN MALE SPRAGUE-DAWLEY RATS TREATED WITH THE VARIOUS CHEMICALS[1]

Type of tumor		Group No. 1 n=29	2 n=29	3 n=28	4 n=30	5 n=29	6 n=29	7 n=30	8 n=30
Kidney:	renal cell tumor	4			2	7	2		
	renal pelvic tumor	1	4(4)[2]		4(2)	3(2)	1(1)		
	nephroblastoma			1					
Urinary bladder: tumor or papillomatosis		5	5		1	4	4	4	2
Liver:	hepatoma				14				
	cystic cholangioma		2			1			
	hemangioma	1							
Nasal cavity: squamous-cell carcinoma		3			1				
Oral cavity: squamous-cell carcinoma		2						1	1
Ear duct: squamous-cell carcinoma		1	1				1	1	
Bulbourethral gl.: squamous-cell carcinoma		1		1					
Forestomach: squamous-cell carcinoma						1	1		
Forestomach: squamous cell-papilloma		2			1	5	1		
Lung: adenocarcinoma		1							
Pancreas: adenocarcinoma							1		
Common bile duct: adenocarcinoma		1							
Small intestine: adenocarcinoma				1		1			
Large intestine: adenocarcinoma		1							
Testes: interstitial-cell tumor			1		1		1		
Retroperitoneum: liposarcoma				1					
Brain:	oligodendroglioma	1							
	meningioma		1						
Adrenal gland: ganglioneuroblastoma			1						
Optic nerve: ganglioneuroblastoma					1				
Skin:	desmoplastic fibroma	4	2	2	1	4		4	1
	hydradenoma		1						
	trichoepithelioma	1							
	lipoma	1						1	
Breast: fibroadenoma			1	1					
Pituitary tumor		2	1		1	6		2	
Sarcoma, unspecified		4	2	1	2	1	1	2	2
Leukemia		2				1			
Total		38	22	8	29	34	13	15	6

[1]Group 1 received phenacetin, group 2 phenazone, group 3 caffeine, group 4 phenacetin, phenazone and caffeine, group 5 phenacetin and phenazone, group 6 phenacetin and caffeine, group 7 paracetamol, and group 8 control diet. – [2]The numbers within brackets indicate invasive renal pelvic tumors.

TABLE IV

THE FREQUENCY OF INFLAMMATORY AND NEOPLASTIC LESIONS IN THE URINARY TRACT IN MALE SPRAGUE-DAWLEY RATS AFTER LONG-TERM TREATMENT WITH TEST CHEMICALS

Group[1]	Renal pelvic tumors	Urothelial hyperplasia of the renal papillae			Pyelitis	Urinary bladder tumors	Urothelial hyperplasia of the bladder mild-moderate	Cystitis and prostatitis
		Mild	Moderate	Severe				
1	1	9	6(2)	11(1)	3	5(2)	3(1)	4
2	4	11(5)	5(3)	3(1)	9	5(2)	5(4)	8
3	–	4	2	1(1)	1	–	2(2)	3
4	4	10(1)	6	7	1	1	–	1
5	3	10(3)	9(1)	6(2)	6	4(1)	2	8
6	1	12(1)	3	6(1)	2	4	4(3)	3
7	–	7	4(4)	6(6)	10	4(4)	6(6)	10
Controls	–	9(6)	1(1)	3(3)	10	2(2)	6(6)	14

[1]Group 1 received phenacetin, group 2 phenazone, group 3 caffeine, group 4 phemacetin, phenazone and caffeine, group 5 phenacetin and phenazone, group 6 phenacetin and caffeine, group 7 paracetamol, group 8 control diet. – [2]Number of rats with signs of infection are shown in brackets.

Pituitary tumors

Pituitary tumors were found in 12 experimental rats, half of them in the group treated with phenacetin and phenazone. The tumors were adenomas, but areas of marked anaplasia and mitotic figures suggestive of focal carcinomatous transformation were seen in 10 rats.

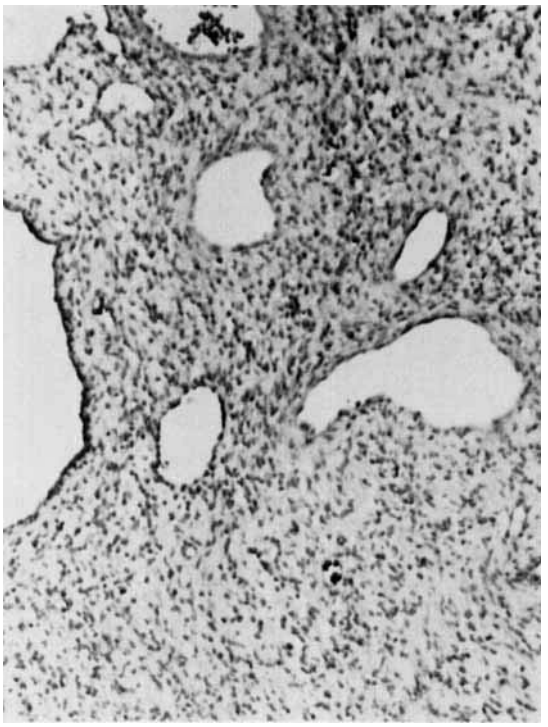

FIGURE 10 — Primitive sarcomatous tumor composed of small cells and containing cyst-like spaces in a phenacetin-treated rat. H. and E., × 120.

Testicular tumors

One testicular tumor in each of groups 2, 4, and 6 was found between 89 and 115 weeks. Histologically the tumors were interstitial-cell tumors. One of the tumors (in group 4) showed mild to moderate cellular and nuclear anaplasia and frequent mitotic figures.

Sarcomas

Sarcomas were found in all groups of rats (Table III). Most of them were undifferentiated, rather pleomorphic spindle-cell sarcomas. In two tumors, giant cells of Touton's type were found, suggestive of histiocytic origin. Two phenacetin-fed rats had rapidly growing sarcomas localized in the head and neck and in the anal region, respectively. These tumors had a rather characteristic appearance and were composed of primitive, small, rather uniform, dark cells with scanty cytoplasm. Within the tumor, cystic areas of varying size were seen (Fig. 10). The tumors were invasive but no metastases were present.

A large retroperitoneal liposarcoma invading the left kidney was detected after 99 weeks in a caffeine-treated rat.

Leukemia

Two phenacetin-fed rats and one treated with phenacetin and phenazone had myelogenous leukemia. The rats were found to have large spleens, kidneys and livers. Histologically these organs as well as lymph-nodes, lungs, myocardium, stomach, urinary bladder and adrenals exhibited dense leukemic infiltrates.

Tumors of nerve tissue origin

Only three nerve tumors were found. One was an oligodendroglioma which was seen at autopsy in a deteriorating phenacetin-treated rat after 64 weeks. The other two were ganglioneuroblastomas. One of these appeared in the optic nerve after 29 weeks in a rat treated with phenacetin, phenazone and caffeine. The other was found in the right adrenal gland after 84 weeks in a phenazone-treated rat.

A number of benign tumors originating in the skin and subcutaneous tissue were also found, particularly among the experimental rats (Table III). The predominating lesion was desmoplastic fibroma, but trichoepithelioma, hydradenoma and lipoma were also seen (Table III).

DISCUSSION

The life-span of the rats differed somewhat in the different groups, being significantly lower among the rats treated with caffeine only. This was due to cardiovascular lesions among these rats, and these findings have been discussed in a separate paper (Johansson, 1981). The mean body-weight at 74 weeks (Table I) was significantly higher among the control rats and the paracetamol-treated rats than in the other groups. The group of rats treated with the combination of phenacetin, phenazone and caffeine had the lowest average body weight. This may imply some toxicity which, however, did not influence life-span. A majority of the control rats and paracetamol-treated rats died from so-called "rat nephrosis", an age-associated lesion of unknown etiology, predominantly found in male Sprague-Dawley rats (Simms and Berg, 1957). However, the possibility of these lesions being induced by high protein content of the diet has recently been considered (Gustavsson, personal communication). Therefore it would seem natural that the group of rats with the highest intake of food would have the highest incidence of rat nephrosis, which was the case in the present study.

Fifteen tumors of the renal parenchyma (cortical renal-cell tumors) were found. The tumors were only found in rats treated with phenacetin alone or in combination with phenazone and caffeine. Spon-

taneous renal-cell tumors of rats are rare (Hard, 1976). The incidence is approximately 0.03% and the present 15 tumors are considered to have been induced probably by a metabolite of phenacetin such as N-hydroxy-phenacetin. Long-term administration of N-hydroxy-phenacetin was associated with the development of renal-cell tumors in rats (Calder *et al.*, 1976) and renal-cell carcinomas have been described in human abusers of analgesics containing phenacetin (Küng, 1976).

Spontaneous renal pelvic tumors in rats seem to be exceedingly rare, only one case being reported in the literature (Hard, 1976). Therefore the 13 renal pelvic tumors in the present study are considered to be induced by the administered chemical. Histologically, eight were urothelial (transitional cell), four were squamous-cell carcinomas and one tumor was a mixed squamous and urothelial carcinoma. The majority of the reported renal pelvic tumors in human analgesic abusers have been urothelial (Bengtsson *et al.*, 1978) but squamous-cell carcinomas have also been reported (Leistenschneider and Ehman, 1973). In the present study phenazone seems more potent than phenacetin for the development of renal pelvic tumors since 11 of the 13 tumors appeared in rats treated with phenazone only, phenacetin, phenazone and caffeine, or phenacetin and phenazone. Only one tumor appeared in a rat treated with phenacetin only, and one in a rat treated with phenacetin and caffeine. However, among the rats treated with phenacetin only, the highest incidence of urothelial hyperplasia of the renal papilla was found (Table IV). This may indicate that phenacetin is a promoter or cocarcinogen. This was also stated by Ito and co-workers (Nakanishi *et al.*, 1978) who found an increased incidence of bladder tumors in rats treated with phenacetin after a sub-carcinogenic dose of BBN [*N*-butyl-*N*-(4-hydroxy-butyl)nitrosamine]. Renal pelvic tumors and urothelial hyperplasia were also found after phenacetin treatment in a previous animal study (Isaka *et al.*, 1979). The majority of human analgesic abusers with renal pelvic tumors had been taking preparations containing phenacetin, phenazone and caffeine, or phenacetin, acetyl-salicylic acid and caffeine, but two cases of renal pelvic tumors have been reported in patients abusing phenazone only (Schabert *et al.*, 1973). However, phenazone is rarely used alone as an analgesic.

Three of the 13 rats with renal pelvic tumors had renal papillary necrosis, in two cases due to invasive growth of the tumor in the renal papilla. Thus it appears that renal papillary necrosis is not a *sine qua non* for the development of renal pelvic tumors in rats treated with phenacetin and phenazone.

Urinary bladder tumors or papillomatosis were most prevalent among the rats treated with phenacetin and phenazone separately or in combination, but were also seen among paracetamol-treated rats and control rats. However, among the latter, the bladder lesions were invariably associated with severe urinary tract infection. Thus the data may indicate that phenacetin and phenazone are weak carcinogens for the lower urinary tract. This is also supported by the study of Isaka *et al.* (1979), who observed epithelial urinary tract tumors in 30% of rats treated with phenacetin. However, they used a dose of 2.5% phenacetin in the diet, which may explain the higher incidence of tumors in their study.

Squamous-cell carcinomas were the most frequent tumors outside the kidneys and urinary tract with the highest incidence (8/20) found among the phenacetin-treated rats. The tumors were mainly localized in the head and neck region (oral cavity, nose and ear duct). Only one squamous-cell carcinoma was found in a control rat. Nasal carcinomas were found in a high proportion of rats after treatment with higher doses of phenacetin in the study by Isaka and co-workers (1979).

All of the forestomach tumors were found in rats treated with phenacetin only or in combination with phenazone and caffeine. Two were carcinomas, the other papillomas. Spontaneous forestomach tumors are exceedingly rare in the rat (Nagayo, 1973), but several carcinogens have been described that induce these tumors (Nagayo, 1973), *e.g.* *N,N'*-2,7-fluorenylenebisacetamide, an aromatic amide (Morris *et al.*, 1961). Phenacetin is also an aromatic amide. It seems most likely that phenacetin or a metabolite of phenacetin is responsible for the development of the forestomach tumors in the present study.

When each group is considered, it is obvious that the groups treated with phenacetin alone or in combination with phenazone, or phenazone and caffeine had a high incidence of malignant or potentially malignant tumors. This is probably a result of the carcinogenic action of phenacetin and phenazone or their metabolites. In the group treated with phenacetin only there were 31 malignant or potentially malignant tumors, in the group treated with phenacetin, phenazone and caffeine, 26, and phenacetin and phenazone, 29. Only five malignant tumors were found among the control rats. I therefore seriously question the use of phenacetin as an analgesic drug, and the presented results indicate the need for additional studies with phenazone. The present study is the first long-term feeding study performed with phenazone, and the effects seem mainly to involve the urinary tract.

Paracetamol demonstrated no significant toxic or carcinogenic effect in the present study. The effects of caffeine on the cardiovascular system have been presented elsewhere (Johansson, 1981), and no evidence for the carcinogenicity of caffeine was observed in the present study.

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