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HISTOLOGICAL CHANGES IN ADRENAL GLANDS OF FEMALE MICE TREATED BY HEPTACHLOR

(Heptaklor Uygulanmış Dişi Farelerin Adrenal Bezlerindeki Hücresel Değişiklikler)

M. Turan AKAY*, Dürdane KOLANKAYA*, K. Çetin ÖZGÜR

INTRODUCTION

The toxicity of various insecticides is of general importance because of their wide use in agriculture. While these pesticides destroy insect or worms, they may have a direct action on nervous, digestive, reproductive and endocrine systems of mammals confined to the treated fields. The hazards of these systems of mammals following consumption of diets containing residues of chlorinated insecticides have been suggested by several reports (VILAR and TULLNER, 1959; DEMATTEIS et al, 1961;MCFARLAND and LACY, 1969; WELCH et al., 1969; WAGNER, 1971; BROOKS, 1974; EROSCHENKO and WILSTON, 1975; FELLEGIOVA et al., 1977). Heptachlor is an insecticide stored mostly in mammalian adipose tissue as epoxide which is more toxic than itself (BROOKS, 1973, 1974). 200 mg/kg of this insecticide produced a significant rise in the concentration of serum glucose and urea and a lowering of hepatic

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glycogen (KACEV and SINGHAL, 1973), As a result of our previous study, although the total glycogen of liver of commercial heptachlor-treated mice was determined a decrease in quantity, it was seen that the total protein was increased (AKAY, 1981). Similar datas were obtained in tissues of chicken embrios given commercial heptachlor (KOLANKAYA, 1979).

The purpose of this investigation was to determine the relation between the decrease of hepatic glycogen of mice treated with commercial heptachlor and histologic changes in adrenal glands.

METHODS AND MATERIALS

In this study, 22-25 g weighed, 3 month old albino mice were used. Three females were kept in metalic individual cages in a room kept at 21,2 ± 1,48°C and 50 ± 4,53 % RH and bedded under natural lighting conditions. Photoperiod was 9 hours during the experiment. This investigation was carried out on 16 albino mice. Ten mice were used as drug-treated group and the rest were used as controls.

Mice were fed with pellet foods. Heptachlor was given with the drinking water which would be obtained 100 ppm of drug. Heptachlor was received from the Research Institute for Plant Protection Chemical and Equipment in Ankara. The percentage of active matter in drug is 89 %. Each of the mice drunk 20 cc of water per day. The experiment went on for 26 days. No mice died during the test. Humudity, food and water supply were always controlled.

At the end of the experiment, adrenal glands from both control and experimental group were excised and fixed in 10 % formol solution. Tissue samples were

embedded in paraffin, sectioned at 7 µm and stained with hematoxylin-eosin (MACMANUS and MOWRY, 1960). The slides were examined with the light microscope and photographed.

RESULTS

The cytoplasm of the cells in the cortex of the adrenal glands of control mice contained sparse lipid droplets which appeared as vacuoles in hematoxylin-eosin prepations. Sinusoidal capillaries coursed between cell groups. The adrenal gland from all heptachlor-fed mice showed a cortical atrophy with the lipid decrease in the cytoplasm of the cells, and a slight hypertrophy was seen in zona glomerulosa after 11^{th} day of the treatment (Fig. 1,2).

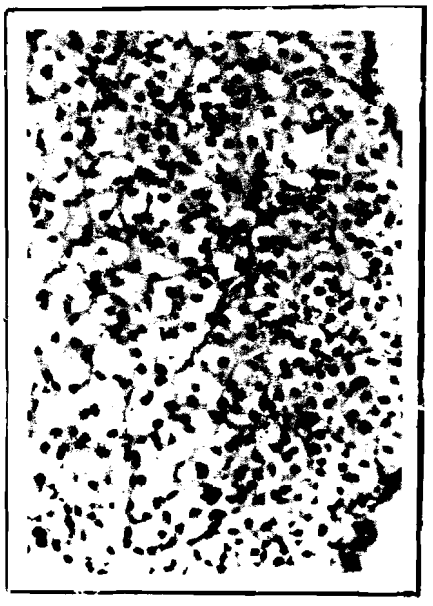

Figure 1: Adrenal cortex of control mice. Magnification x 250

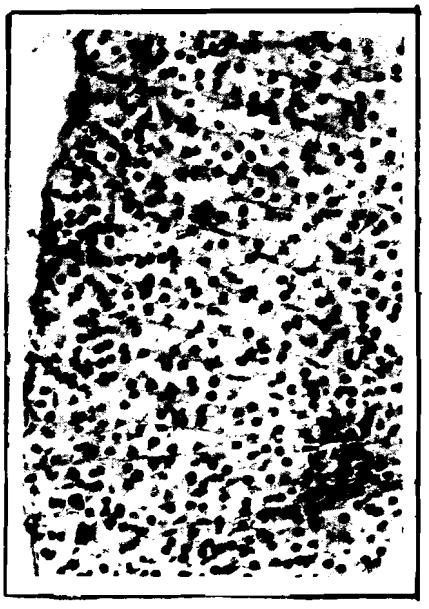

Figure 2: Adrenal cortex of mice treated with 100 ppm of heptachlor (11^{th} day of treatment). Magnification x250

Hypertrophy of the cortical cells was characteristic of all heptachlor-treated mice with a heavy lipid accumulations and presence of granulation in 26th day of the treatment. Adrenal cortex showed more congestion, cell degeneration with extensive destruction and fibrosis by the effect of insecticide used at the end of the experiment (Fig. 3).

Figure 3: Fibrosis and cell degeneration in adrenal cortex of mice treated with 100 ppm of heptachlor (26th day of treatment) Magnification x 250

DISCUSSION

Biological effects of the insecticides can be revealed by studying their influence on the endocrine systems. The results of our present study showed that histological changes in the adrenal glands of the mice were caused by the direct effect of the commercial heptachlor. A cortical atrophy was determined in the 11th day of treatment since the lipid in cytoplasm of cortical cells mobilized for energy source needed to detoxificate the insecticide during the beginning of experiment. Nelson and Woodward (1949), showed that 0,p'DDD from chlorinated

insecticides produced atrophy of the cortex area of adrenal glands in the dog. Besides this, kepon caused hypertrophy in cortex and medulla of adrenal glands of both sex of quail (EROSCHENKO and WILSTON, 1975). In the present investigation the hypertrophic cortical cells indicate their hyperfunction with the possible increase of the glucocorticoid hormones secretion into general circulation. Some organochlorine compounds have similar effects on the extraadrenal metabolism of glucocorticoid hormons (CUETO et al., 1958; VILAR and TULLNER, 1959). The increase of these hormons caused the decrease of hepatic glycogen and the increase of serum glucose (ANDAÇ et al.,1977). Hepatic glycogen decreased in mice treated with commercial heptachlor (AKAY, 1981). Bergenstal et al. (1960) showed the presence of extensive destruction and fibrosis of the adrenal cortex in a patient who received 0,p'DDD and suffered for adrenocortical cancer. The cell degeneration and at last the fibrosis in our preparations can also show that commercial heptachlor can cause cancer when treated for a long time.

ACKNOWLEDGMENTS

Laboratory assistance was provided by Mr. S.Çalış whose help is greatly appreciated.

ÖZET

Klorlu hidrokarbon insektisitlerden ticari heptaklorun 100 ppm subletal dozu, dişi albino farelere oral yolla 26 gün uygulanmış ve ilacın fare adrenal bezlerine olan etkisi histolojik olarak araştırılmıştır. Sonuçlar heptaklorun adrenal bezlerde artan salgı faaliyetivle birlikte kortikal hücre dejenerasyonuna, hipertrofiye ve fibrosise yol açtığını göstermiştir.

SUMMARY

The sublethal dose, 100 ppm of commercial heptachlor from chlorinated insecticides was applied orally to the female albino mice for 26 days and the effect of it on adrenal glands was investigated histologically. The results indicate that heptachlor caused cortical cell degeneration, hypertophy and fibrosis with increased secretory activity in adrenal glands.

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EFFECT OF EXOGENOUS cAMP ON THE MOTILITY OF FASCIOLA HEPATICA

(*Fasciola hepatica* hareketleri üzerine ekzojen cAMP'nin etkisi)

Sami Aydoğan*, Aşkın Tümer*, Ahmet Noyan*

ÖZET: Bu çalışmada cAMP ve dibutryl-cAMP'nin karaciğer sülüğü olarak bilinen *Fasciola hepatica*'nın hareketleri üzerine etkisi incelendi. Mezbahada yeni kesilen kovun ve sığırlardan alınan *F. hepatica*'lar laboratuvara getirilerek 37°C deki çeşitli ortamlarda (izotonik salin çözeltisi, safra, tirod çözeltisi gibi) hareketleri gözlendi. Serotonin'in *F. hepatica*'nın hareketlerini hızlandırdığı saptandı. Ancak değişik derişimlerde kullanılan ekzojen cAMP ve dibutryl-cAMP *F. hepatica*'nın gözlenen hareketleri üzerine bir etki göstermedi.

INTRODUCTION

F. hepatica, also known as liver fluke, frequently appears among the cattle all around the country and causes serious economical loss every year. This parasite lives in the bile ducts and can stay there for a few years. *F. hepatica* also occupies an important place in regard with the experiments with cAMP as it is the

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first non-mammalian with adenly cyclase activity. Mansour and his collegues (MANSOUR et al, 1960; STONE and MANSOUR, 1967) suggested that cAMP activity was stimulated by serotonin. cAMP has also been implicated in the post-synaptic response to several neurotransmitters, including serotonin (GREENGARD, 1976). In addition, it was also suggested that serotonin and cAMP, added in the homogenates of this parasite, enhanced phosphofructokinase (an enzyme which controls glucolysis in these organisms) activity (MANSOUR and MANSOUR, 1962). In another study it was suggested that cAMP possibly mediates the motility enhancement effect of serotonin on *F. hepatica* (MANSOUR, 1957 and ROBISON et al, 1971). However, it is still unknown whether exogenous cAMP has an effect on the motility of the liver fluke or not.

In this study, we examined the effect of various concentrations of cAMP and dibutryl-cAMP (assuming that it penetrates the cell membrane more easily than cAMP) on the motility of *F. hepatica*.

MATERIALS AND METHODS

The liver flukes were obtained from recently slaughtered calves and sheep. The parasites were brought to the loboratory in a Krebs solution and were placed in 2 ml of various solutions and their motilities were observed under a stereoscopic microscope for 10 minutes. The solutions were; saline (0,9 % NaCl), bile, saline + bile (1:1) and tyrode solution. All the solutions were kept at 37^{o}C during the observations. The effect of serotonin

(10^{-5}M) and of cAMP (10^{-1}, 10^{-2}, 10^{-3} and 2 x 10^{-3}M Adenosine 3':5'-Cyclic Monophoshoric Acid Sodium salt) and dibutryl-cAMP were tested in each of these solutions mentioned above. Additionally, in order to facilitate the diffusion of cAMP into the organism, 3% and 6% solutions of dimethylsulfoxyde (DMSO) were used in some experiments.

RESULTS AND DISCUSSION

Under normal conditions (in isotonic saline, pH 7.4) two different types of motility were observed in the liver fluke. One of these was a contraction-relexation activity of the whole body with a period of 30-35 seconds and the second was a fluctation of the edges of the body.

It was first observed that serotonin-free, cAMP-free and dibutryl-cAMP-free solutions and DMSO itself had no effect on the motility. Serotonin (10^{-5}M) caused a marked enhancement in the both types of the motility. The contractions and relexations increased 2-3 fold and the frequencies of these movements have gone up to 0.10-0.12 seconds^{-1}.

In the case of four different concentrations of cAMP and dibutryl-cAMP we did not observe any change in the motilities. Addition of DMSO (3% and 6%) also did not show any effect. These results are represented in Table 1.

Table 1. Effects of different mediums on the motility of *F.hepatica*.

Number of parasites	Medium	Activation of the Motility
10	Serotonin (10^{-5}M)	+ + +
10	cAMP (10^{-1}M)	-
10	cAMP (10^{-2}M)	-
10	cAMP (10^{-3}M)	-
10	cAMP ($2x10^{-3}$M)	-
10	dibutryl cAMP (10^{-1}M)	-
5	dibutryl cAMP (10^{-3}M)	-
5	cAMP (10^{-1}M)+DMSO (%3,%6)	-
5	dibutryl cAMP (10^{-1}M)+DMSO (%3,%6)	-

It was concluded that exogenous cAMP and dibutryl-cAMP had no enhancing effect on the motility of *F. hepatica* while serotonin alone had a marked effect and caused a 2-3 fold acceleration in the motilities.

Therefore in contrast to the suggestion of Mansour (1957), Robison et al (1971) and Greengard (1976), who has suggested that a serotonin mediated increase in cAMP might be responsible for the increased neurotransmitter release and accompanied behavioural sensitisation in certain invertebrate nerve networks, we could not find any evidence supporting that cAMP mediates the effect of serotonin on the motility of *F. hepatica*.

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SYSTEMATICAL RESEARCHS ON THE SAKARYA BASIN FISHES (Pisces) (SAKARYA HAVZASINDA YAŞAYAN BALIKLAR (Pisces) ÜZERİNE SİSTEMATİK ARAŞTIRMALAR)*

Füsun Erk`akan** ,Mustafa Kuru**

SUMMARY

In this research, 40 species and 12 supspecies which belong to 11 families have been identified. Of these, 7 species and 2 subspecies are new for the Sakarya basin, 2 species are new for Anatolia.

INTRODUCTION

Especially in recent years, research on the fishes has become very important in Turkey. Up till now, the important part of the freshwater fauna of Turkey has been determined (KARAMAN, 1969; HANKO, 1924; TANYOLAÇ, 1968). The fishes that are called Danubian fauna elements,prefer stagnant waters, have deep and strongly compressed bodies. These fishes are known as far east as Terme-Bafra region in the Black sea basin (KURU, 1972). Hence, our research area takes place in the distribution area of these fishes. On the other hand, the Sakarya basin is a transit area for fishes of Europe origin. The fishes of the Sakarya basin were studied in order to have more fully document about the fish species of this area.

MATERIALS AND METHODS

Specimens were collected using seines (experimental gill net, Frammel net and otter trawl) and electroshocker from different water systems of the Sakarya basin

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showing different ecological characteristics. The electro-shoker consists of a 220 volt, 500 watt, A.C. generator and electrodes. This generator is small and light, it can be carried in a back-pack. Electrodes may be fashioned from a square foot of galvanized iron or copper laminate with 1.0 cm. or 1.5 cm. square mesh, attached to a frame of wood. In this method, electrical current is applied to water, thus immobilizing the fishes. The fishes can then be collected easily by the investigator.

The collected fishes are placed in the nylon bags which contains 4 % formalin, carried to the laboratory and preserved in 70 % alcohol for further systematic examinations.

RESULTS

As will be seen from the fallowing list, 40 species and 12 subspecies belonging to 11 families were found in the Sakarya basin.

I. Fam. CLUPEIDAE
Alosa (Caspialosa) pontica EICHWALD, 1838

II.Fam. SALMONIDAE
Salmo trutta macrostigma (A. DUMERIL, 1858)

III.Fam. ESOCIDAE
Esox lucius LINNAEUS, 1758

IV. Fam. CYPRINIDAE
Rutilus rutilus (LINNAEUS, 1758)
Leuciscus (Squalius) cephalus (LINNAEUS, 1758)
Leuciscus (Squalius) borysthenicus (KESSLER, 1859)
Phoxinus phoxinus LINNAEUS, 1758
Scardinius erythrophthalmus (LINNAEUS, 1758)
Aspius aspius taeniatus (EICHWALD, 1831)
Tinca tinca (LINNAEUS, 1758)
Chondrostoma nasus (LINNAEUS, 1758)

Gobio gobio (LINNAEUS, 1758)
Barbus plebejus lacerta HECKEL, 1843
Barbus plebejus escherichi STEINDACHNER, 1897
Chalcalburnus chalcoides derjugini (BERG, 1923)
Alburnus alburnus (LINNAEUS, 1758)
Alburnus orontis SAUVAGE, 1882
Alburnoides bipunctatus fasciatus (NORDMANN,1840)
Alburnoides bipunctatus eichwaldi(FILIPPI,1863)
Blicca bjoerkna (LINNAEUS, 1758)
Abramis brama (LINNAEUS, 1758)
Vimba vimba tenella (NORDMANN, 1840)
Rhodeus sericeus amarus (BLOCH, 1782)
Carassius carassius (LINNAEUS, 1758)
Cyprinus carpio (LINNAEUS, 1758)
Capoeta tinca (HECKEL, 1843)
Capoeta capoeta sieboldi (STEINDACHNER, 1864)

V. Fam. COBITIDAE
Cobitis taenia LINNAEUS, 1758
Cobitis simplicispinna HANKO, 1924
Cobitis (Sabanejewia) aurata (FILIPPI, 1865)
Neomacheilus angorae angorae STEINDACHNER,1897
Neomacheilus angorae bureschi DRENSKY, 1928
Neomacheilus lendli HANKO, 1924

VI. Fam. SILURIDAE
Silurus glanis LINNAEUS, 1758

VII. Fam. SYNGNATHIDAE
Syngnathus nigrolineatus EICHWALD, 1851

VIII.Fam. CYPRINODONTIDAE
Aphanius chantrei GAILLARD, 1895

IX. Fam. MUGILIDAE
Mugil cephalus LINNAEUS, 1758

X. Fam. PERCIDAE
Perca fluviatilis LINNAEUS, 1758
Lucioperca lucioperca (LINNAEUS, 1758

XI. Fam. GOBIIDAE

Proterorhinus mormoratus (PALLAS, 1811)
Gobius (Neogobius) fluviatilis (PALLAS, 1811)
Gobius (Babka) gymnotrachelus (KESSLER, 1857)
Gobius (Ponticola) syrman (NORDMANN, 1840)

Alburnoides bipunctatus eichwaldi (FILIPPI, 1863). New record for the Sakarya basin.

The Pharyngeal teeth of *Alburnoides bipunctatus fasciatus* are always 2.5-4.2 and these subspecies are present in the Sakarya basin (SLASTENENKO, 1955-56, BERG, 1964 and KURU, 1975). But the pharyngeal teeth of *Alburnoides bipunctatus eichwaldi* are always 2.5-5.2 and this fish lives in the Kura-Arax system (KURU, 1980). The number of pharyngeal teeth are used in the keys to distinguish these two subspecies from each other. Some of our specimens caught from the Sakarya basin are similar to of *A. bipunctatus eichwaldi*. by means of pharyngeal teeth.

Aphanius chantrei GAILLARD, 1895. New record for the Sakarya basin.

According to AKŞIRAY (1948), the speciment collected from Sakarya-Çifteler were identified as *Aph. anatolias*. However, our materials from different localities of Sakarya basin, Sakarya-Çifteler, Eminekin-Çifteler, Porsuk stream-Harmandalı-Eskişehir, Kaymaz and Sarısu-Gordion show the same characteristics of *Aphanius chantrei*, with the exception of dark speckles on the anal fin of the male specimens.

Barbus plebejus lacerta HECKEL, 1843

Some *Barbus* specimens from the Sakarya basin were identified by HANKO (1924) as *B. p. lacerta*. According to the revision of Turkish *Barbus* species by KARAMAN (1971), only *Barbus plebejus escherichi* lives in this

region. Some of our Barbus specimens from the Sakarya basin were identifed as *Barbus plebejus lacerta* because of the same systematical characteristics. Gill rakers of these subspecies vary from 6 to 9 (KARAMAN, 1971), but in the Sakarya basin the variation is from 8 to 16.

Cobitis (Sabanejewia) aurata FILIPPI, 1865, New record for the Sakarya basin.

All of the specimens caught from the Dinsiz stream near Adapazarı are close to *Cobitis aurata* since they have more or less developed dermal crests between D. and A., 10-15 dark spots on the sides of the body, and strong suborbital spines. But differs from *Cobitis aurata* with slightly emerginate Caudal fin and a big, dark rounded spot at the top of the Caudal base.

Rutilus rutilus, Carassius carassius, Blicca bjoerkna, Gobius (Babka) gymnotrachalus and *Chalcalburnus chalcoides* are also new records of the Sakarya basin and there are no differences from descriptions by earlier authors.

Gobius (Ponticola) syrman (NORDMANN, 1840) New record for Anatolia.

Some specimens caught from the upper reaches of Devrek and Gerede streams (2.000 m. altitude) are very similar to *Gobius syrman* but differ from it in some systematical characteristics, such as: Caudal peduncle more than half as long as deep, four light and three dark transverse band on D_1, I or II unbranched rays in D_2, lips more broadened and ventral, base of Pectoral fins, occipital region, one-fourth of the operculum covered with cycloid scales, throat is naked.

Phoxinus phoxinus LINNAEUS,1758 New record for Anatolia.

Phoxinus phoxinus cholhicus lives in Western Transcaucasica and Thrace (BERG, 1964 and KURU, 1980). Specimens caught from the Yenidağ and Yassıgeçit streams for the first time in Anatolia, differ from *Phoxinus phoxinus cholhicus* in having a small scaled area on abdomen before Ventrals, maximum depth of the body is 5.0-5.5 times in standard length, caudal peduncle length is 1.6-2.0 times in minimum body depth.

According to the descriptions of the earlier authors (SLASTENENKO, 1955-56, BERG, 1964 and KURU, 1975), the Dorsal fin of *Capoeta tinca* has three unbranched rays. This number is four, however, for some specimens which were caught in our research area.

According to KARAMAN (1969), *Capoeta capoeta sieboldi* is distributed in the basin of Sakarya, Kızılırmak, Çoruh and the lateral line of these subspecies varies from 50 to 59, there are tubercules at the mandibular symhysis, the upper lips are fimbriated, the number of gill rakers on the first arch is between 25-30. In our material, we found differences in the number of scales in lateral line, varying from 60 to 72, among specimens caught from various stations. On the other hand, there are no tubercules at the mandibular symphysis, the upper lips are generally not fimbriated and the number of gill rakers on the first arch is between 20-26. In this case, because of the similarities existing between the lateral line, our specimens resemble *C . capoeta bergamae,* but differ from this subspecies in the number of gill rakers on the first arch, mouth structure and length of the barbels. Because of these reasons, our specimens have been identified as *C. capoeta sieboldi*.

Some specimens of *Leuciscus cephalus*, caught from the Enne Dam-Kütahya, have some variations in the number of branched rays in the Dorsal and Anal fins(D.III/(6) 7-8;A.III/(5) 6-8). On the other hand, the number of gill rakers on the first arch of these speciemens are increased to 13 in the Sakarya basin. According to SLASTENENKO (1955-56). BERG (1964) and KURU (1975), however, this number is between 8-10.

Some meristic and morphometric characters of *Alburnus orontis* which is widely distributed in our region show certain important variations. Pharyngeal teeth of this species are 2.5-5.2, 2.5-4.1, 1.5-4.2, 2.5-4.2 , 1.5-5.1 and 1.5-4.1. On the other hand, keel between ventral and anal fins scaled. Pharyngeal teeth of all specimens, previously caught, were determined as generally 2.5-5.2 but sometimes as 2.5-4.2, 2.5-4.1, 1.5-5.1 and keel between the ventral and anal fins is scaleless (SLASTENENKO, 1955-56).

But, the number of gill rakers on the first arch of *Alburnus alburnus* is between 17-22 and the number of the branched rays in the anal fin varies from 14 to 17. Because of these similarities, the genus of *Alburnus* is very close to *Chalcalburnus* (BERG, 1964). Thus, systematical characteristics used to distinguish these two genera from each other are unsuccessful. After our studies, we have concluded that the status of *Alburnus* and *Chalcalburnus* must be re-examined.

In our studies on *Cobitidae* species, we determined certain difficulties in some systematical characteristics used to distinguish species up till now.

Before, some Cobitis specimens having unbranched suborbital spine under each eye determined as *C. simlicispinna* by HANKO (1924) and some others having bifid suborbital spines under each eye determined as *C. taenia* from the Sakarya basin (SLASTENENKO-1955-56). But, some specimens in our collection coming from Pazar disjunction, Kızılcahaman, Hamam stream-Çeltikçi and Numanoluk-Seyitgazi have one unbranched suborbital spine under one eye and one branched bifid suborbital spine under the other eye. Because of this feature, these specimens can be thought of as hybrids, between *C. simlicispinna* and *C. taenia*, but they are more abundant than *C. simlicispinna*. On the other hand, some of their systematical characteristics are close to *C. taenia*.

Some *Noemacheilus* specimens determined from the Sakarya basin earlier have different systematical characteristics from *N. angorae* and *N. löndli* (BANARESCU, NALBANT, 1964). Species of this genus differ from each other with the shape of the intestine and bony capsule arround the swimbladder, coloration, mouth structure and some meristic characters (BANARESCU, 1964). Specimens caught from Bolu-Seben show the same characteristics as *N. tigris* living in the Euphrates-Tigris basin. There are some *N. tigris* specimens collected by KURU (1975) in the Zoology museum of Hacettepe University. Thus, the specimens from the Sakarya and Euphrates-Tigris basins are compared with each other. Subsequently, we found that except for body depth, all other systematical characteristics of Sakarya basin specimens are similar to those of *N. tigris*. However, because of the difficulties in distinguishing the species of this genus, these and some other specimens from the Sakarya basin have not been examined in this study.

The identifications of species of this family is very difficult and therefore systematical studies (BANARESCU-1964) of them for Turkey have been unsuccessful. For this reason, the systematical status of this family must be re-examined with more specimens by using morphometric and meristic methods.

DISCUSSION

From these, *Barbus plebejus lacerta, Rutilus rutilus, Carassius carassius, Blicca bjoerkna, Chalcalburnus chalcoides, Aphanius chantrei, Gobius (Babka) gymnotrachelus* which have not been found in the Sakarya basin, *Phoxinus phoxinus* and *Gobius syrman* which have not been found in Anatolia before were identified. Gobius syrman which is a sea fish was found in the upper reaches of Devrek and Gerede streams. *Cobitis (Sabanejewia) aurata* which was thought to live only in Eastearn Anatolia and *A. bipunctatus.eichwaldi* which was thought to live in Kura-Arax system, were found also in the Sakarva basin. *Aphanius analotias* which was found previously in the upper reaches of Sakarya River-Çifteler was identified as *Aphanius chantrei*. Some systematical variations of *Leuciscus cephalus, Capoeta tinca, Capoeta capoeta sieboldi, Alburnus orontis, Barbus plebejus lacerta* such as the number of fin rays, scales of lateral line, gill rakers on the first arch and pharyngeal teeth were obtained, It is impossible to distinguish the species of *Cobitidae* according to the keys given previously. On the basis of these results, we believe that it is necessary to re-examine this family.

ÖZET

Bu araştırmada, Sakarya havzasında yaşayan 11 familyaya ait 40 tür ve 12 alt tür saptanmıştır. Bunlardan 7 tür ve 2 alt tür Sakarya havzası için, 2 tür ise Anadolu için yeni kayıttır.

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CHROMATOGRAPHIC ANALYSES OF MICROBIAL FAT FROM INDUSTRIAL WASTES

Nevin KESKİN* Ali MATUR*

SUMMARY

In order to produce lipids and fatty acids via yeasts, by the fruit wastes that don't have economical value and cause environmental pollution were added to the media. Different lipids were determined qualitatively by thin-layer chromatography (T.L.C.), from the microorganisms grown on morello cherry waste, grape waste, and molasse media. Qualities of the lipids obtained from these wastemedia were not different from the lipids obtained from the control glucose medium.

By using Gas-liquid chromatography (G.L.C.) a technique used for quantitative analyses of fatty acids, different kinds and amount of fatty acids obtained from the waste-media were found same as of the fatty acids obtained from the control medium.

INTRODUCTION

The demand for oils and fats both for edible and technical purposes, continiues to increase. However fat production from microorganisms may become feasible. If the starting materials are cheap, the oils produced from them are also cheap. The quality of the oil being produced must be optimised.

Yeasts, would seem the most likely candidates for bio-oil producing microorganisms, since the product should be at a high concentration and non-toxic (Woodbine, 1959,.Enebo and Ivamoto, 1966., Kessel, 1968.,Ratledge, 1968 a.,Ratledge, 1970).

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Oil producing microorganisms can utilize a wide variety of carbon sources such as glucose and other pure sugars and n-alkanes. Among these, mostly n-alkanes used for production of bio-oil as n-alkanes are widely available and relatively cheap (Bos and Boer, 1968., Ratledge, 1968 a,b.,Ratledge, 1970.,Thorpe and Ratledge, 1972., Chenouda and Jwanny, 1972).

In this study, the microbial fat production from the industrial wastes which would cause environmental pollution is studied.

MATERIALS AND METHODS

Candida albicans CBS 562 and C.albicans 628 were used in this study.

As a control, the medium described by Murray and Walker (1956) was used. Instead of an expensive growth factor biotin, a by-product of an alcohol industry was added into this medium. The pH was adjusted to 5.5 with NaOH.

Medium with morello cherry waste: 500 gr dry morello cherry waste was dissolved in 1,5 lt distilled water for overnight and then filtered. Chemical ingrediends in Murray-Walker (1956) medium were added to this filtrate (except of $(NH_4)_2SO_4$ and glucose). The amount of C and N in the filtrate was examined and then used for the preparation of the medium.

Medium with grape waste: It was prepared like morello cherry waste medium.

Medium with molasse: Molasse which was used contained 45 % Carbonhydrate (C.H). The medium prepared contained 1 % C.H. in addition, 1 gr/lt $(NH_4)_2SO_4$ was added to the medium.

Batch cultivation was carried out in 250 ml erlenmayer flasks containing 100 ml media in a rotary shaker at 30°C and 150 rpm. for 3 days (66 hrs). This period was determined by previous studies (Keskin,1981).

Lipids were extracted according to Dawson and Craig (1966). The extracted lipids were analysed by thin-layer chromatography (T.L.C) on silica gel G (stahl) for qualitative analyses. The lipids were separated by using methanol : chloroform : water (65: 25:4, by vol) solvent system (Wagner et al, 1961). Spots were detected by various spray reagents. Glycolopids were detected by spraying diphenylamine reagent (Jatzkewitz and Mehl,1969), phospholipids by a molybdenum blue reagent (Dittmer and Lester, 1964). Cholin containing phospholipids by Dragendroff reagent (Wagner et al, 1961), and a ninhydrin spray was used to detect lipids having a free-amino group (Skipski et al, 1962).

Gas-liquid chromatography (G.L.C) was used for quantitative analyses of the fatty acids. Fatty acids were extracted according to Ratledge (1968 a) and trans-methylated with benzene methanol conc. H_2SO_4(10:20:1,by vol) for 2 hrs (Nichols et al, 1965). The methyl esters of fatty acids separated by gas chromatography using a Varian 3700 gas chromatography equiped with an integrator, with a column containing 20 % diethylene glycol succinate on chromosorb W (Mc Nair and Bonelli, 1969). N_2 as carrier gas was at 40 ml/min. and H_2 was at 30 ml/min.

RESULTS

C.albicans CBS 562 and C.albicans 628 were grown on glucose medium, morello cherry waste medium, grape waste medium, and molasse medium for 3 days (66 hrs). Then cells were harvested and lipid extracted were

analyzed by T.L.C. (Figures 1,2,3,4,5,6,7,8).

C.albicans 562 and C.albicans 628 were incubated on above media except molasse medium for 3 days, then cells were harvested and fatty acids were obtained and transmethylated. Fatty acid methyl esters were analysed by G.L.C. (Figures 9,10,11, 12, 13,14). As seen in figures the carbon source was used and transformed to fatty acids by yeast in both glucose and fruit waste medium. The percentages of fatty acids are shown in Table I.

DISCUSSION

Lipid extracted from C.albicans CBS 562 and C.albicans 628 were analysed by T.L.C for the qualitative examinations. Various lipids were detected by spraying various reagents. In both glucose and fruit waste medium the lipids observed were not qualitatively different. Wagner et al (1961) and Dawson and Craig (1962) have separated lipids by T.L.C. using various spray reagents. Marinetti (1962) reported detection of lipids by chromatography on silicic acid impregnated paper.

Kates and Baxter (1962) have shown that, C.lipolytica produced fatty acids with C_{16-18} and found oleic acid at high level (47,5 %). Ratledge and Saxton (1968) and Hall and Ratledge (1977) have also observed C_{14-24} fatty acids and they found the oleic acid at a high level (39 % and 39,6 %). We found, C.albicans CBS 562 and C.albicans 628 produced fatty acids with C_{8-21} on glucose and fruit waste media and observed oleic acid at a high level (app. 30 %). Linoleic (18:2) and linolenic acid (18:3) which are essential fatty acids were higherin fruit waste medium then glucose medium. Ratledge (1970), Thorpe and Ratledge (1972), have also found n-alkanes

are more suitable for the fatty acid production especially for linoleic acid . The quality of the fatty acids we achieved on the fruit wastes are similar to that which are essential in daily use.

ÖZET

Endüstriyel atık sayılan ve çevre kirlenmesine neden olan meyve suyu atıkları yağ üretmek amacı ile üreme ortamlarına eklendi. Vişne küspeli, üzüm küspeli ve melaslı ortamlarda üretilen mikroorganizmalardan çeşitli lipidlerin elde edilebileceği T.L.C. ile kalitatif olarak ortaya kondu. Atıklı ortamlardan elde edilen lipidlerin, kontrol ortamı olarak kullanılan glukozlu ortamlardakinden kalitatif olarak farklı olmadığı belirlendi.

Yağ asitlerinin kantitatif olarak belirlendiği G.L.C. çalışmalarında da meyve atıklı ortamlardan elde edilen yağ asitleri çeşit ve miktarlarının kontrol ortamına (Glukozla) benzer sonuçlar verdiği saptandı.

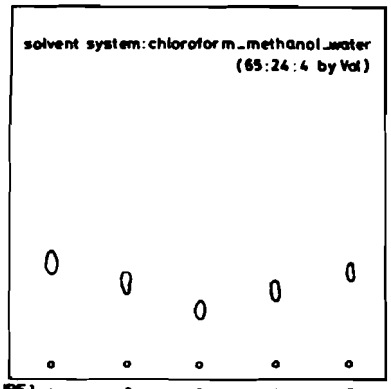

FIGURE:1 Rf: 1 0.350, 2 0.285, 3 0.191, 4 0.254, 5 0.318

1_PHOSPHOLIPIDS from C.albicans 628 grown on glucose media.
2_PHOSPHOLIPIDS from C.albicans CBS 562 grown on morello cherry waste media.
3_PHOSPHOLIPIDS from C.albicans 628 grown on morello cherry waste media.
4_PHOSPHOLIPIDS from C.albicans CBS 562 grown on grape waste media.
5_PHOSPHOLIPIDS from C.albicans 628 grown on grape waste media.

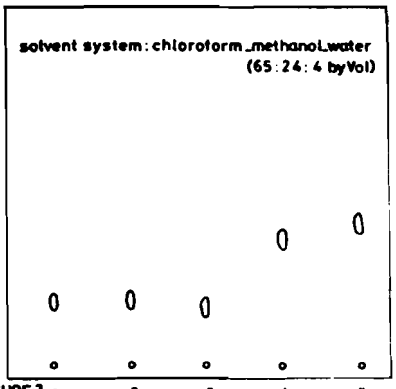

FIGURE:2 Rf: 1 0.148, 2 0.196, 3 0.159, 4 0.398, 5 0.429

1_LIPIDS HAVING A FREE AMINE GROUP from C.albicans 628 grown on glucose media.
2_LIPIDS HAVING A FREE AMINE GROUP from C.albicans CBS 562 grown on morello cherry waste media.
3_LIPIDS HAVING A FREE AMINE GROUP from C.albicans 628 grown on morello cherry waste media.
4_LIPIDS HAVING A FREE AMINE GROUP from C.albicans CBS 562 grown on grape waste media.
5_LIPIDS HAVING A FREE AMINE GROUP from C.albicans 628 grown on grape waste media.

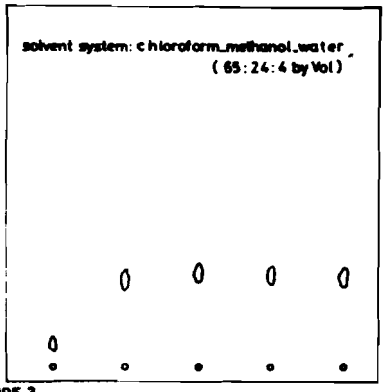

FIGURE:3 Rf: 1 0.065, 2 0.294, 3 0.326, 4 0.326, 5 0.314

1_GLYCOLIPIDS from C.albicans 628 grown on glucose media.
2_GLYCOLIPIDS from C.albicans CBS 562 grown on morello cherry waste media.
3_GLYCOLIPIDS from C.albicans 628 grown on morello cherry waste media.
4_GLYCOLIPIDS from C.albicans CBS 562 grown on grape waste media.
5_GLYCOLIPIDS from C.albicans 628 grown on grape waste media.

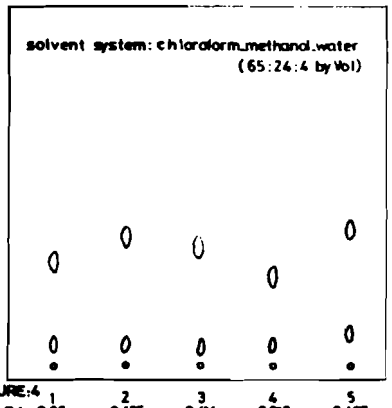

FIGURE:4 Rf: 1 0.35, 2 0.426, 3 0.414, 4 0.318, 5 0.458

1_CHOLIN CONTAINING PHOSPHOLIPIDS from C.albicans 628 grown on glucose media.
2_CHOLIN CONTAINING PHOSPHOLIPIDS from C.albicans CBS 562 grown on morello cherry waste media.
3_CHOLIN CONTAINING PHOSPHOLIPIDS from C.albicans 628 grown on morello cherry waste media.
4_CHOLIN CONTAINING PHOSPHOLIPIDS from C.albicans CBS 562 grown on grape waste media.
5_CHOLIN CONTAINING PHOSPHOLIPIDS from C.albicans 628 grown on grape waste media.

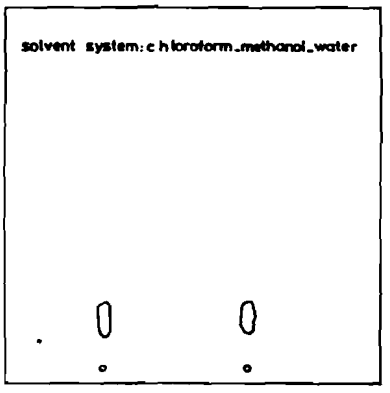

FIGURE: 5

	1	2
Rf:	0.147	0.164

1_CHOLIN CONTAINING PHOSPHOLIPIDS from C.albicans CBS 562 grown on molasse media.
2_CHOLIN CONTAINING PHOSPHOLIPIDS from C.albicans 628 grown on molasse media.

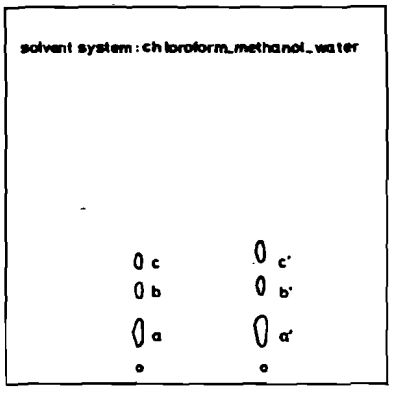

FIGURE: 6

	1	2
Rf:	a: 0.148	a': 0.167
	b: 0.277	b': 0.277
	c: 0.380	c': 0.380

1_LIPIDS HAVING A FREE AMINE GROUP from C.albicans CBS 562 grown on molasse media.
2_LIPIDS HAVING A FREE AMINE GROUP from C.albicans 628 grown on molasse media.

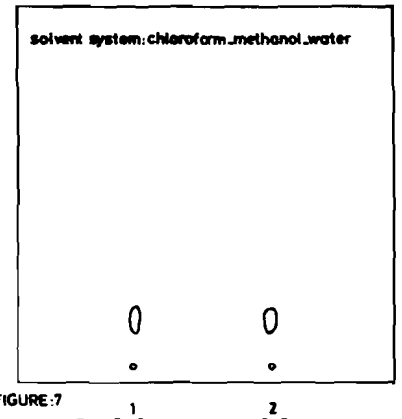

FIGURE: 7

	1	2
Rf.	0.15	0.15

1_PHOSPHOLIPIDS from C.albicans CBS 562 grown on molasse media.
2_PHOSPHOLIPIDS from C.albicans 628 grown on molasse media.

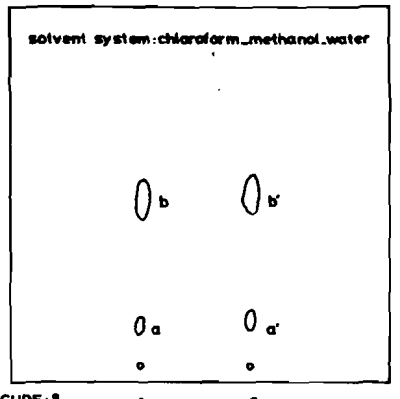

FIGURE: 8

	1	2
Rf.	0.118 (a)	0.147 (a')
	0.517 (b)	0.529 (b')

1_GLYCOLIPIDS from C.albicans CBS 562 grown on molasse media.
2_GLYCOLIPIDS from C.albicans 628 grown on molasse media.

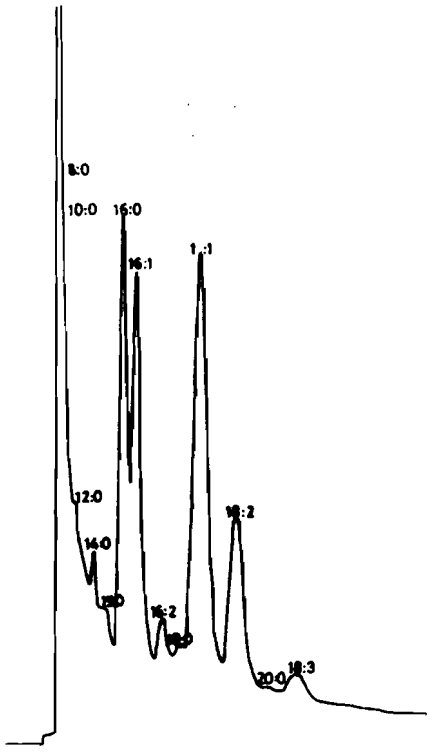

Figure 9 : Gas liquid chromatography traces of C. albicans CBS 562 grown on glucose medium

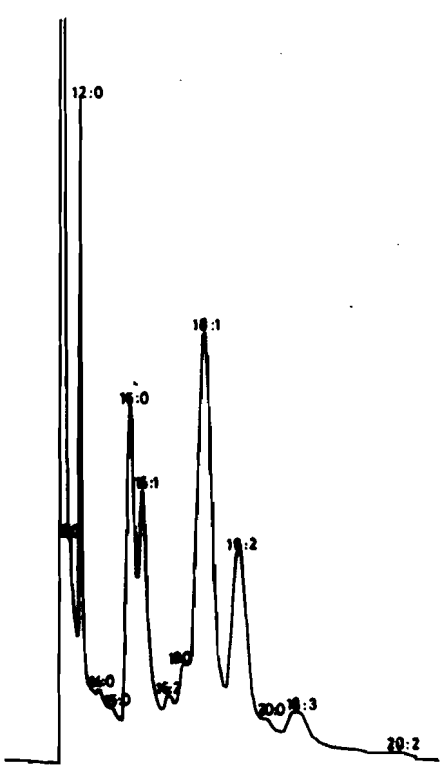

Figure 10 : Gas-liquid chromatography traces of C. albicans 620 grown on glucose medium.

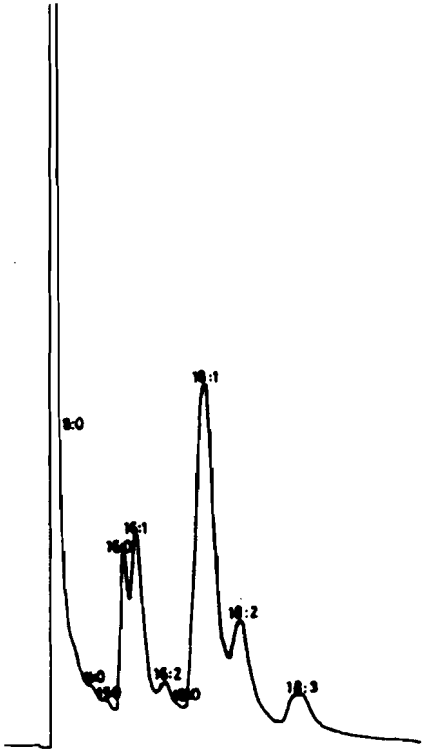

Figure 11 : Gas-liquid chromatography traces of C. albicans CBS 562 grown on morello cherry waste medium

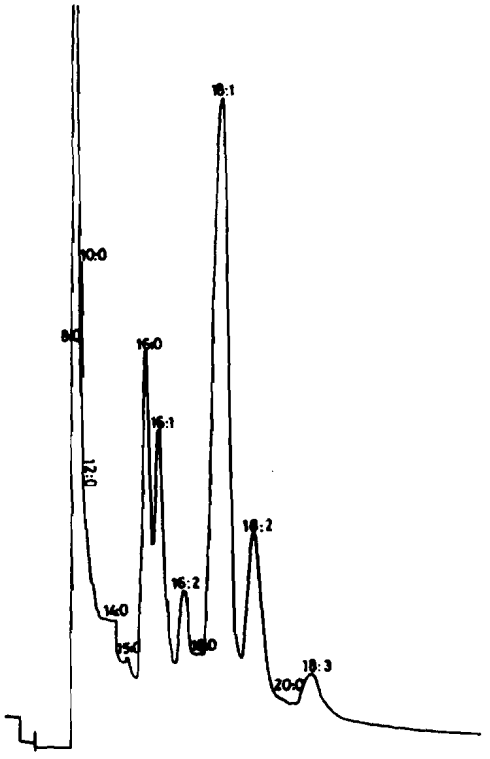

Figure 12. Gas_liquid chromatography traces of C.albicans 628 grown on morello cherry waste medium.

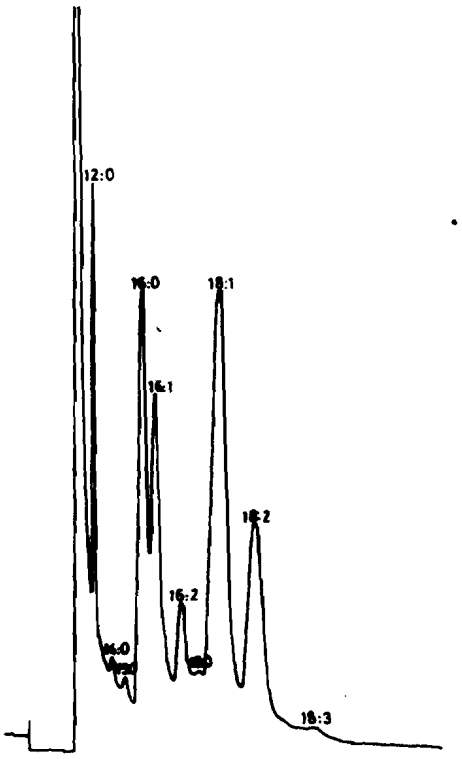

Figure 13 : Gas_liquid chromatography trace of C.albicans CBS 562 grown on grape waste medium.

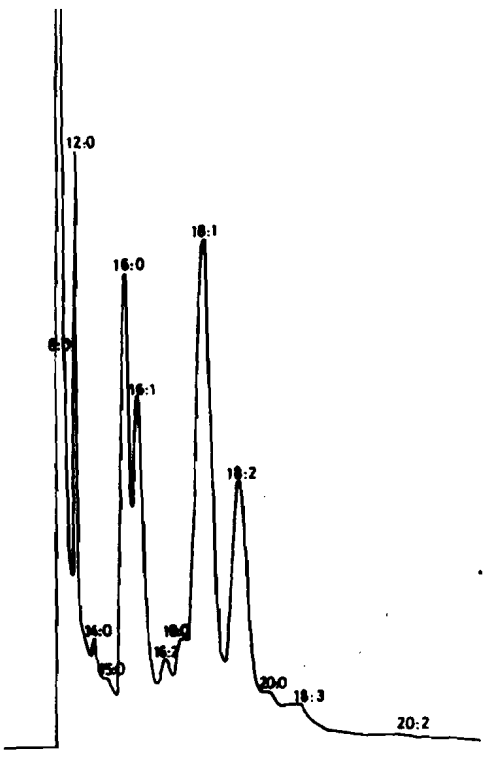

Figure 14 : Gas_liquid chromatography trace of C.albicans 628 grown on grape waste medium.

Table I : Percentage of Fatty Acids from C.albicans CBS 562 and C.albicans 628 grown on various media.

Media	Microorganism	PH	Fatty Acids according to the number of C atoms. 8:0	10:0	12:0	14:0	15:0	16:0	16:1	16:2	17:0	18:0	18:1	18:2	18:3	20:0	20:2	21:0	% of Unsaturated fatty acids
Glucose media	C.albicans CBS 562	5.5	5.18	13.01	7.87	5.20	4.27	15.86	17.90	4.2	–	2.69	26.68	12.37	4.1	0.73	–	–	65.3
	C.albicans 628	5.5	0.06	–	11.49	3.16	2.1	12.33	12.99	2.84	–	4.17	28.52	14.9	5.18	1.26	0.86	–	65.4
Morello cherry waste media	C.albicans CBS 562	5.5	?	–	–	3.1	3.36	8.55	14.22	4.46	–	?	36.64	12.86	6.8	–	–	–	74
	C.albicans 628	5.5	2.26	5.29	?	2.28	2.7	10.51	11.74	5.46	–	1.86	34.12	14.69	7.48	?	–	–	68.5
Grape waste media	C.albicans CBS 562	5.5	–	–	10.04	3.14	2.83	14.55	15.19	6.49	–	2.74	27.93	15.22	3.85	–	–	–	66.7
	C.albicans 628	5.5	0.298	–	10.286	3.45	12.154	13.892	12.514	3.161	–	3.92	28.29	15.76	5.48	1.72	0.58	–	65.2

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IN VITRO AND IN VIVO INHIBITION OF MICE BRAIN ACETYLCHOLINESTERASE BY SOME CHLORINATED HYDROCARBON INSECTISIDES

(Bazı Organoklorlu İnsektisitlerle Fare Beyni Asetilkolinesterazının in vitro ve in vivo inhibisyonu)

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SUMMARY

Mouse brain acetylcholinesterase inhibition by Heptachlor, Endosulfan, BHC and DDT was investigated in vitro and in vivo. It was determined that concentration of 0,005 ppm Heptachlor, 0,01 ppm Endosulfan, 0,05 ppm BHC and 0,1 ppm DDT inhibited acetylcholinesterase in vitro. This inhibition was depended on doses of drugs. The inhibition of acetylcholinesterase that increased depending on the period in the brain of pregnant mice when treated with 150 ppm/day dose of insecticide was observed. It was showed that Heptachlor had more toxic effect on nervous system than Endosulfan and others in vitro and in vivo.

INTRODUCTION

Chlorinated hydrocarbon insecticides are widely used to kill insects. In the view of their mode of action, these insecticides were not clearly described. DDT and its toxic relatives inhibite enzymes of oxidadive phosphorylation (Brooks, 1974). This inhibition is related to the basic action of DDT.

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The signs of poisoning both in insects and mammals indicated an action of DDT and analogs on the nervous system. Treated insects and mice rapidly became hypersensitive to external stimuli and developed tremors of the body and appendages. After a period of violent motion, they fell on their backs and the continuous leg movements eventually became more spasmodic due to paralysis (Winteringham and Barnes, 1955; Akay and Alp, 1981). Similar findings were observed on chick embryos, too (Kolankaya and Şişli, 1979). The symptoms in mammals resulted from disturbances in the central nervous system where the motor area of the cerebrum and the cerebellum appeared to be involved since they were said to be a direct correlations between the severity of both acute and chronic clinical toxicity in the adult rat and the concentrations of drugs in the brain (Holan, 1969; Dale at al , 1963). DDT poisoning in American cockroaches were reported to result in the accumulation of acetyhcholine in the nervous system at the prostrate stage but these appeared to have no inhibition of cholinesterase in the neurocord of this insect in vitro or in other preparations examined (Metcalf, 1955; O'Brien, 1967).

The purpose of this investigation was to determine the relationship between the defect of central nervous system and paralyses with acetylholinesterase inhibition in vitro and in vivo after treatment with insecticides on mice.

MATERIALS AND METHODS

In this study, 22-25 g weighed, 3 month old pregnant albino mice were used. Commercial BHCα-isomer,DDT, Endosulfan and Heptachlor treated as 150 ppm per day with food on which mice were fed for 3 weeks. All of the

insecticides were received from Production Center of Agricultural Drugs and Exuipment in Ankara. Heptachlor,DDT (86 %),α-isomer of BHC (99 %) and Endosulfan (94 %) were used for in vitro study in the concentrations of 0,005, 0,01, 0,02, 0,03, 0,04, 0,05, 0,1 0,2, 0,3, 0,4 ppm.

The brains were taken out from mice and homogenated in distilled water. Brain homogenates were used as a source of enzyme. The enzyme activity was measured by Ellman method using acetylthiocholin as substrate (Ellman an Courtney, 1961).

RESULTS AND DISCUSSION

I. In vitro study:

The activities of acetycholinesterase (AchE) showed decrease which were dose-depended by the effect of chlorinated hydrocarbon insecticide. The decrease of enzyme

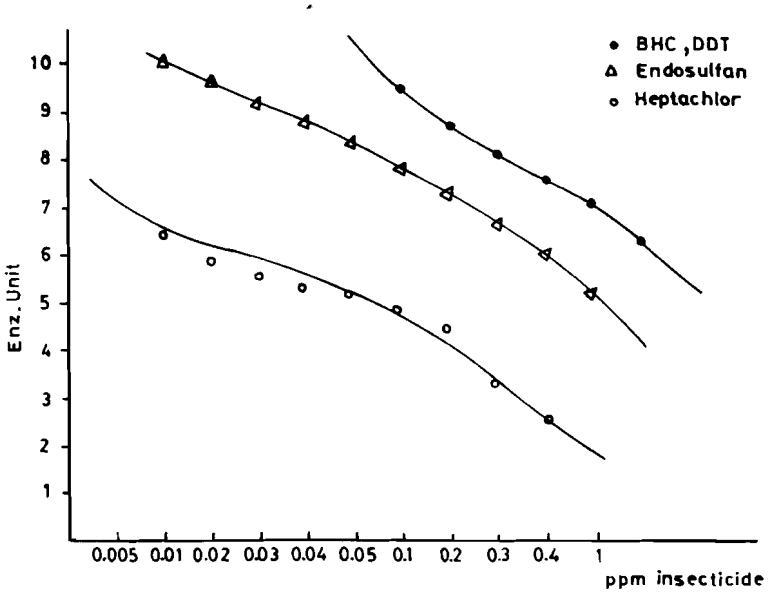

Figure 1- In vitro inhibition of acetylcholinesterase by BHC, DDT, Endosulfan and Heptachlor in mouse brain.

activity was different for each insecticides, such as 0,005 ppm of Heptachlor, 0,02 ppm Endosulfan and 0,1 ppm of BHC and DDT caused a decrease in enzyme activity (Figure 1).

II. In vivo study:

The α-isomer of commercial BHC, DDT, Heptachlor and Endosulfan that treated with food caused inhibition of acetylcholinesterase enzyme in pregnant albino mouse brain. Heptachlor was more effective than other insecticides. Decrease of enzyme activity began at 1. week and continued for 3. week (Figure 2). The enzyme activity of control groups showed a decrease in first week and continued during the second week, however, the activity in test group showed a decrease untill the end of the gestation.

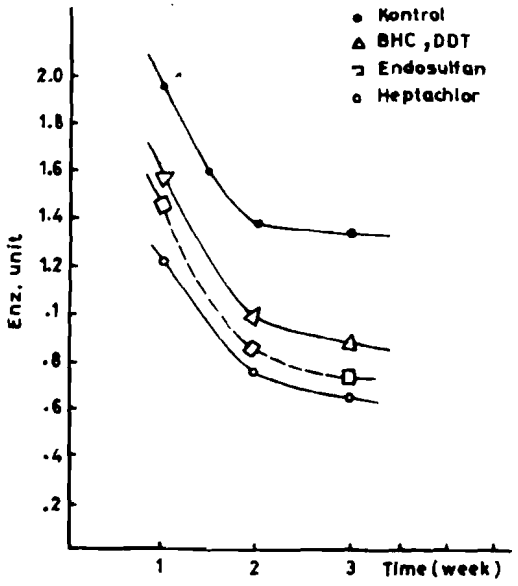

Figure 2- In vivo inhibition of acetylcholinesterase by BHC, DDT, Endosulfan and Heptachlor in pregnant mouse brain.

Various concentration of chlorinated hydrocarbons caused inhibition of mice brain acetylcholinesterase in vitro. But the effect of inhibition of each insecticide differ in concentrations. Brooks (1974) reported that DDT did not inhibit acetyhcholinesterase in American cockroaches but caused an accumulation of acetylcholine in the nervous system at the prostrate stage. We showed that DDT and other chlorinated hydrocarbon insecticides inhibited acetylcholinesterase in brain tissue, but DDT concentration was higher as 0,1 ppm than the other insecticides. Lower concentration of DDT showed no inhibition of cholinesterase. DDT,α-BHC, Endosulfan and Heptachlor inhibited brain acetylcholinesterase both invivo and in vitro, but heptachlor was more effective on asetylcholinesterase enzyme. This result showed that heptachlor was more toxic effect on nervous system than Endosulfan and α-isomer of BHC. Because of the effect of gestation on the nervous system, acetyhcholinesterase activity of control groups appeared to decrease at the beginning of gestation. After one week, the enzyme activity was kept at the same degree until the end of gestation. At the same time, chlorinated hydrocarbon insecticides inhibited oxidative metabolism enzymes such as sucsinicoxidase, cytochrome oxidase and carbonic anhydrase in the mammals (O'Brien, 1967; Brooks and Harrison, 1972).

Chlorinated hydrocarbon insectisides inhibited both acetylcholinesterase and ATP-ase in the nerve membrane of insects (Koch et al , 1969). The inhibition of ATP-ase breaks down the Na^{+}, K^{+}, Mg^{+2} and Ca^{+2} equilibrium on the nerve membrane. Because of this, the impulse conduction is broken in nerve membrane,as a result of paralysis (Matsumura and Patil, 1969). When ATP-ase

inhibition is together with acetylcholinesterase inhibition, they both cause irreversible paralysis in the organism.We observed this type of tetani and paralysis in hen embryo and albino mice by the effects of BHC, DDT, Heptachlor (Kolankaya and Şişli, 1979; Akay and Alp, 1981).

ÖZET

Heptaklor, Endosülfan, BHC ve DDT'nin fare beyni asetilkolinesteraz enziminin inhibisyonuna etkisi in vitro ve in vivo çalışıldı. Heptaklorun 0,005 ppm'lik, Endosülfanın 0,01 ppm'lik, BHC'nin 0,05 ppm'lik ve DDT'nin 0,1 ppm'lik dozunun inhibisyona neden olduğu in vitro gösterildi. Bu inhibisyonun doza bağlı olarak arttığı saptandı. Yiyecekle 150 ppm günlük insektisit dozu gebe farelere uygulandığında beyinde zamana bağlı olarak artan asetilkolinesteraz inhibisyonu gözlendi. Heptaklorun in vivo ve in vitro çalışmalarda sinir sistemi üzerine endosülfan ve diğerlerinden daha toksik etkiye sahip olduğu gösterildi.

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