Ecology and Epidemiology Studies of Dengue Viruses in Din Daeng, Bangkok, Thailand

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OBJECTIVES :

1. To determine the seasonal incidence of apparent and inapparent dengue virus infections in Bangkok adults and children.

2. To establish the duration and magnitude of the antibody response to primary and secondary arbovirus infection, including dengue virus type 1, 2, 3, 4 and Japanese encephalitis (JE) virus.

3. To assess experimentally the ability of wild *A. aegypti* to serve as a vector of dengue viruses on a seasonal basis.

4. To determine the seasonal prevalence rate of dengue viruses in adult and immature *A. aegypti* populations.

5. To determine the population density of the wild *A. aegypti* population on a seasonal basis and seasonal availability and utilization of artificial containers by this species for oviposition.

BACKGROUND : Epidemiological and ecological investigations have shown that dengue viruses are endemic in Bangkok and that the primary vector and vertebrate host are *Aedes aegypti* and man, respectively (1, 2). Apparent human infections occur throughout the year. However, a marked increase in the incidence of illness has been observed during the rainy season (June - September), and age specific attack rate data have placed children 15 years of age or less at greatest risk (2). Early findings indicated that the variation in the incidence of infection was related to the population density of *A. aegypti* (3). More recent data suggest that the magnitude of change in absolute population size of

A. aegypti in Bangkok was not large enough to explain the seasonal fluctuations in the incidence of apparent dengue virus infection (4). The latter investigation was extended to consider the longevity and blood feeding patterns of *A. aegypti*. Data did not show seasonal variation in 24 hour survival; however, biting rates appeared to vary as indicated by a marked decrease during the cool season of the year (5). According to the authors, the decrease in biting rates and a possible increase in the length of the extrinsic incubation period of the vector may cause the decrease in the incidence of dengue virus infections in man during this part of the year.

Except for the rainy season, data relative to the seasonal prevalence rates of dengue viruses in the *A. aegypti* population of Bangkok is lacking. In addition, no consideration has been given to the vector competence of this species during the different seasons of the year. Available data have shown that temperature affected the degree of susceptibility of mosquito to infection with arboviruses and the time required before transmission of arbovirus was accomplished by bite (6, 7). These factors and the possibility of seasonal changes in longevity, biting activity, etc. of *A. aegypti* may prove to influence the seasonal variation in prevalence of dengue viruses as well as the apparent variation of the circulation of different dengue virus serotypes in the mosquito and human population.

Transovarial transmission of arboviruses in their respective natural mosquito vectors has been proven for some California encephalitis group viruses (8, 9). Recent experimental findings have shown that dengue viruses and JE virus can be transmitted transovarially by *Aedes albopictus* (10, 11). Evidence of transovarial transmission of dengue viruses in natural populations of mosquitoes has not been reported.

Estimates of *A. aegypti* population density employing human biting and landing counts, sweep net, aspiration-vacuum sweep of resting adults, and dipping collections for larvae and pupae are biased by a number of individual human traits dependent on the collectors (12). Other collecting techniques, developed to avoid these problems include the pyrethrin knockdown, oviposition traps and the one larvae per container techniques. The former test is biased by space, timing, and sealing problems, and a reluctance of the collectors to work in the presence of pyrethrin aerosol spray. The latter two techniques are better tools, but, they cannot be employed alone for population density estimates.

METHODS :

Study area : The study area for this investigation was a section of the Din Daeng area of Bangkok (Figure 1). The area is circumscribed by Prachasongkro Road on the north, Soi Charasongkhro on the west, Din Daeng I Road on the east and Klong Sam Sen on the south. Included in the area are 20 four-floor apartment buildings, many two-three storey shop-houses, approximately 4 acres of confluent single storey slum dwellings and a few single or two storey residential homes. The total human population of the study area was between 13,000 to 14,000.

Census of the population and mapping of the area : One hundred families of the Din Daeng study area that had a child attending Phibounprachasan School were randomly selected for the study. Each family was interviewed regarding address, income level, occupation, family size, ages, general health, and cultural and behavioral practices possibly related to disease recognition. Interviews of the families and the mosquito habitat surveys were conducted concurrently and on a seasonal basis. The entire area was mapped in regard to layout of housing, streets and other permanent landmarks.

Dengue virus infection of the human population : The seasonal incidence of dengue, Chikungunya, and JE infections was based on seroconversion rates determined by hemagglutination inhibition tests (13). Blood was obtained from family members before and after each season of the year. Overt dengue virus infections were determined by bi-weekly visits to the residence of each family in the study population. Classification of apparent and inapparent infection was based on previously defined criteria (14, 15).

Seasonal assessment of the vectorial capacity of *A. aegypti* : *A. aegypti* larvae were collected from wild populations within the Din Daeng study area. Adult mosquitoes reared from the larvae were allowed to ingest graded doses of dengue virus to establish the threshold level of infection. The extrinsic incubation period was determined by allowing individual mosquitoes to ingest normal blood from a hanging drop suspension at intervals after the mosquitoes ingested dengue viruses (16). An aliquot of the remaining blood suspension was assayed to determine if a particular mosquito secreted virus while feeding. Assay of blood and mosquitoes for virus was performed by plaque assay employing LLC-Mk_2 cells (17). Temperature and humidity were monitored continuously during the experiments and were maintained similar to that of the study area.

Seasonal dengue virus prevalence rates in *A. aegypti* : Adult *A. aegypti* and immature stages of this species will be assayed for virus by the mosquito inoculation technique employing *Toxorhynchites splendens* as a bioassay host (18).

Survey of mosquito larval habitats : The availability of *A. aegypti* larval habitats and the utilization of these sites by this species was determined during each season of the year. Residences of the 100 families in the study were surveyed indoors and outdoors to determine the total number of potential and positive larval breeding sites. A definition for containers inside or outside of houses was determined on the basis of a roof or roof-like structure above the container(s). Lids may occur on containers in both categories; however, if rainwater could flow or fall into the containers, then it was considered to be outside. Each container was searched thoroughly employing a flashlight and a 4 oz suction syringe for *A. aegypti* larvae and pupae.

A standardized water container (ong jar) was placed in the residence of each of the 100 families selected for study. These jars will serve to provide estimates of the population density of *A. aegypti* obtained through concurrent employment of different, complementary sampling techniques. Each sampling device is being designed to focus on a certain life stage, behavioral factor, and/or physiological state of the mosquito population. The sampling methods (traps) described below are being designed to eliminate the bias of the human traits listed above and will be employed, provided preliminary tests show them

to be reliable and effective sampling devices.

A floating larval trap will be used to determine the population density of *A. aegypti* larvae and pupae. These traps will be used on a rotating schedule throughout the 100 family units and collections will be made during one 24 hour period each week.

Emergence cone traps that fit over the mouth of water jars will be used to determine adult emergence patterns and densities during each season of the year. Gravid adults captured in these trap will be separated from the newly emerged mosquitoes and will be used for virus isolation studies.

An estimate of the oviposition rate of *A. aegypti* will be determined seasonally by estimating the number of gravid females. This will be determined by capturing gravid females in a specially designed trap that captures the female, but does not allow her to reach a substrate for oviposition. The gravid condition of trapped females will be confirmed by microscopic examination and gravid females will be saved for virus isolation attempts.

Additional sampling for estimating the adult density will be attempted by using a suction type trap. This trap will be designed to sample active flying adults, or by attracting them to a resting site. All females trapped by this method will be assayed for virus.

RESULTS : To assess the possible error because of biased sampling of the adult population of Din Daeng, (e.g. those adults who are always at home have a greater chance of being sampled) the serum specimens collected from adults in the Din Daeng area during the dry season survey were tested for arboviral antibody. Of 94 adults, ranging in age from 19 to 70, 92(98%) had evidence of flavivirus infection and 74(79%) had evidence of alphavirus infection. Because of these very high rates of seropositivity and a lack of male/female difference or age-dependent variation, the adult sample was considered adequate. All other antibody determinations are in progress.

The development of techniques for vector competence studies was hampered by the refusal of *A. aegypti* to ingest virus-blood suspensions. Subsequent experiments in which the virus-blood suspensions were supplemented with 10% sucrose yielded a 90 to 100% feeding rate. Preliminary results indicated that this technique was effective for infecting colony *A. aegypti* with dengue virus types 1, 2 and 3. Ten to 20% of the mosquitoes became infected after ingesting 4.0 $\log_{10}$ $SMICLD_{50}$/1.0 ml of dengue virus type 1. After ingesting 6.0 $\log_{10}$ $SMICLD_{50}$ of the same virus type, the infection rate increased to 60%. Approximately 20% of the *A. aegypti* became infected by employing a dose of 6.5 $\log_{10}$ $SMICLD_{50}$ of dengue virus type 2. A similar dose of dengue virus type 3 yielded an infection rate of 60 to 90%. *A. aegypti* failed to become infected after ingesting 5.0 $\log_{10}$ $SMICLD_{50}$ of dengue virus type 4. Attempts to demonstrate transmission of dengue viruses by infected *A. aegypti* were not successful. Studies are in progress to assess the capability of *A. aegypti* of the Din Daeng study area to serve as a vector of dengue viruses during different seasons of the year and to correlate vector competence of *A. aegypti* populations with the genetic composition of this species. A description and the results of studies to develop techniques for establishing the genetics of *A. aegypti* populations are reported in

the Entomology Section of this Annual Progress Report.

A. aegypti larvae were collected for transovarial transmission studies from the premises of 50 houses of dengue hemorrhagic fever patients in Bangkok. A total of 531 pools consisting of 13,005 larvae were collected from 1 November, 1977 through 20 March, 1978. Attempts to isolate dengue virus from 172 pools (4,280 larvae) were unsuccessful. Of the 172 pools, 100 were assayed by direct and delayed plaque technique and by the mosquito inoculation technique in conjunction with fluorescent antibody assay. The other pools were assayed by the latter technique. Attempts to isolate dengue viruses from two pools of 25 females and four pools of 42 male *A. aegypti* reared from field collected larvae were unsuccessful.

The number of *A. aegypti* larvae collected during each month and the average number of larvae obtained from each house is presented in Table 1. *A. aegypti* larvae were found in 76.6% (49/64) of the houses. Of 12,318 of the total 13,005 larvae collected, 32.8% and 67.2% were collected from containers located inside and outside of the house, respectively. *A. aegypti* larvae collected inside houses were obtained from water jars located in bathrooms (45%), water in kitchens (22%), water jars in bedrooms (13%) and the remainder were found in water jars in unspecified locations. Ninety five percent of the larvae collected outside houses were found in water jars and 5% were obtained from claypots, tin cans, tires and ant-traps. The average number of *A. aegypti* collected per house was 203; however, the density of the larvae population was associated with a decrease in the number of larvae occurring in both inside and outside breeding containers. Containers of 20 houses were sampled a second time during January, February and March 1978. *A. aegypti* larvae were present in all houses; however, the number of larvae collected was 1,768 compared to 3,706 larvae obtained during November and December, 1977. Virological and ecological investigations of *A. aegypti* larvae will continue but the studies will be conducted in the Din Daeng study area as described in this report.

Investigations of the seasonal availability and utilization of artificial containers by *A. aegypti* in the Din Daeng study area began in April 1978. The residence of each of the 100 families was surveyed inside and outside during the dry season, 3-18 April, and the early wet season, 30 May - 15 June. Natural containers were not found during either survey, as vegetation is practically non-existent in this densely packed urban area. The most common containers with water found in the residences were clay ceramic water (ong) jars, ant traps, flower vases, tin cans, plastic buckets and pans, and cement water basins. A seasonal comparison of the containers with water and those with *A. aegypti* is shown in Table 2. Initially, "containers with water" was considered a valuable index, however, the subjectiveness of deciding which container should be counted (numerous plastic wash basins, buckets, etc. that are used daily) has made accurate counts nearly impossible. Consequently, "containers with *A. aegypti*" is considered to be the most valuable index. As shown in Tables 2 to 4, there was a considerable increase in the number of *A. aegypti* positive containers, both inside and outside houses, between the dry season and the early wet season. In addition, houses positive for *A. aegypti* larvae increased from 24% during the dry season to 43% for the early wet season.

The seasonal prevalence of *A. aegypti* larvae and pupae inside and outside houses of different residential types is summarized in Tables 5 and 6. These data demonstrated an increase in *A. aegypti* positive containers both inside and outside of houses between the dry season and the early wet season. The only residential type not affected by the seasonal increase in water available to the family units was the high rise-flats for outside containers. Figure 2 depicts the monthly precipitation levels as recorded by the Bangkok Meteorological Station. The slum residences yielded the highest number of *A. aegypti* positive containers and family houses during both seasons. However, shop-house residences had the highest percentage of inside positivity for *A. aegypti* during the early wet season. These data suggest that the slum residences maintain the largest reservoir of *A. aegypti* through the dry season.

An inexpensive larval trap designed to float on the water surface in water jars has been developed and tested (Table 8). This circular trap is made of clear plastic and measures approximately 13 cm in diameter and 13-14 cm in depth. The trap is designed such that larvae moving vertically toward the surface of the water are captured by passing through the aperture of the apex of a cone that leasts into the collecting container. The data suggest that this trap is a highly efficient collector of *A. aegypti* and *Culex quinquefasciatus* larvae and/or pupae (Tables 7 and 8). Although the trap is free of human collecting bias, the high returns in just 24 hours, suggest that the trap is biased, probably because larvae congregate around floating objects.

Three different types of mosquito emergence traps, two types of oviposition container traps, and four modified electric suction traps (without light) have been developed and partially tested. Thus far, the tests on the emergence, oviposition and electric suction traps have proven inconclusive. Development and testing of traps for sampling *A. aegypti* densities will continue. The larval trap is scheduled to be placed in the residence of the 100 family units in October 1978 and to be used as a routine sampling device thereafter. After reliable sampling techniques are developed, investigation will be initiated to consider the remaining objectives of this study.

Table 1. *Aedes aegypti* larvae obtained from the premises of houses of dengue hemorrhagic fever patients during 1977 and 1978 in Bangkok, Thailand

Date	Houses		*Aedes aegypti*		Total Larvae	Ave. Number Larvae/House
	Larvae	No Larvae	Outside	Inside		
Nov 77	10	00	5558	2504	8,062	806
Dec 77	10	00	1295	1097	2,392	239
Jan 78	16	02	1193	575	1,768	098
Feb 78	08	04	431	47	478	040
Mar 78	05	09	256	49	305	022
	49/64(76.6%)	15/64(23.4%)	8733(67.2%)	4272(32.8%)	13,005	203

Table 2. Seasonal utilization of containers for 100 houses by *Aedes aegypti* larvae and/or pupae, Din Daeng, Bangkok, Thailand

Date of Surveys	Containers with Water				Percentage of Containers with *A. aegypti*		Percentage of Houses Positive for *A. aegypti*
	Larvae and/or Pupae		No Larvae or Pupae				
	Inside	Outside	Inside	Outside	Inside	Outside	
3-18 April, 1978 (Dry Season)	55	11	532	68	9.4(55/587)	13.9(11/79)	29
30 May-15 June (Early Wet Season)	86	24	489	72	15.0(86/575)	25.0(24/96)	43

Table 3. Seasonal change in utilization of containers inside 100 family dwellings* by *Aedes aegypti* larvae and/or pupae, Din Daeng, Bangkok, Thailand

Date of Survey	*A. aegypti* Larvae and/or Pupae			% Change (+) from Dry Season		
	Containers	Dwellings	Container Dwelling	Containers	Dwellings	Container Dwelling
3-18 April 78 (Dry Season)	55	25	2.20	-	-	-
30 May-15 June 78 (Early Wet Season)	86	37	2.32	+56	+48	+5

* Same family dwellings used during both surveys

Table 4. Seasonal change in utilization of containers outside 100 family dwellings* by *Aedes aegypti* larvae and/or pupae, Din Daeng, Bangkok, Thailand

Date of Survey	*A. aegypti* Larvae and/or Pupae			% Change (+) from Dry Season		
	Containers	Dwellings	Container Dwelling	Containers	Dwellings	Container Dwelling
3-18 April, 78 (Dry Season)	11	8	1.37	-	-	-
30 May-15 June, 78 (Early Wet Season)	24	14	1.71	+118	+75	+25

* Same family dwellings used during both surveys

Table 5. The seasonal utilization of containers inside 100 houses* by *Aedes aegypti* larvae and/or pupae for different types of residences and the number of larvae and/or pupae pooled for virus isolation studies

Residential Type	% of Units	Dry Season				Early Wet Season			
		Positive for *aegypti*			# Larvae** & Pupae	Positive for *aegypti*			# Larvae** & Pupae Pooled
		Con-tainers	Units	Cont./ Unit		Con-tainers	Units	Cont./ Unit	
Highrise Flats	44	9	2	4.50	118	15	6	2.50	189
Slums	36	33	15	2.20	417	40	18	2.22	406
Shop or House	20	13	8	1.63	164	31	13	2.38	420
Total	100	55	25	2.20	699	86	37	2.32	1,015

* The same family units used during both surveys
** Larvae and pupae for virus isolation studies

Table 6. The seasonal utilization of containers outside 100 houses* by *Aedes aegypti* larvae and/or pupae for different types of residences and the number of larvae and/or pupae pooled for virus isolation studies

Residential Type	% of Units	Dry Season				Early Wet Season			
		Positive for *aegypti*			# Larvae** & Pupae	Positive for *aegypti*			# Larvae** & Pupae Pooled
		Con-tainers	Units	Cont./ Unit		Con-tainers	Units	Cont./ Unit	
Highrise Flats	44	0	0	0	0	0	0	0	0
Slums	36	10	7	1.43	153	19	12	1.58	224
Shop or House	20	1	1	1.00	20	5	2	2.50	70
Total	100	11	8	1.37	173	24	14	1.71	294

* The same family units used during both surveys
** Larvae and pupae for virus isolation studies

Table 7. *Aedes aegypti* larvae captured in traps during a 24 hour period

Trials*	Date Test Ends	Larvae Trapped Per Container					Captured	
		Trap 1	Trap 2	Trap 3	Trap 4	Trap 5	Total	%
1	4 Aug	24	56	61	70	48	259	51.8
2	8 Aug	31	59	39	18	40	187	37.4
3	11 Aug	57	74	80	67	67	345	69.0
4	16 Aug	69	61	58	56	23	267	53.4
5	18 Aug	67	64	72	45	62	310	62.0
Total		248	314	310	256	240	1,368	54.7

Table 8. *Culex quinquefasciatus* larvae captured in larvae traps during a 24 hour period

Trials*	Date Test Ends	Larvae Trapped Per Container					Captured	
		Trap 1	Trap 2	Trap 3	Trap 4	Trap 5	Total	%
1	4 Aug	71	54	62	74	24	285	57.0
2	8 Aug	47	30	71	67	64	279	55.8
3	11 Aug	56	27	52	88	60	283	56.6
4	16 Aug	62	40	84	75	85	346	69.2
5	18 Aug	65	76	65	43	60	309	61.8
Total		301	227	334	347	293	1,502	60.1

* Each trial tested 5 replicates, each involving one trap per each 27 liter water jar and 100 fourth stage larvae per jar.

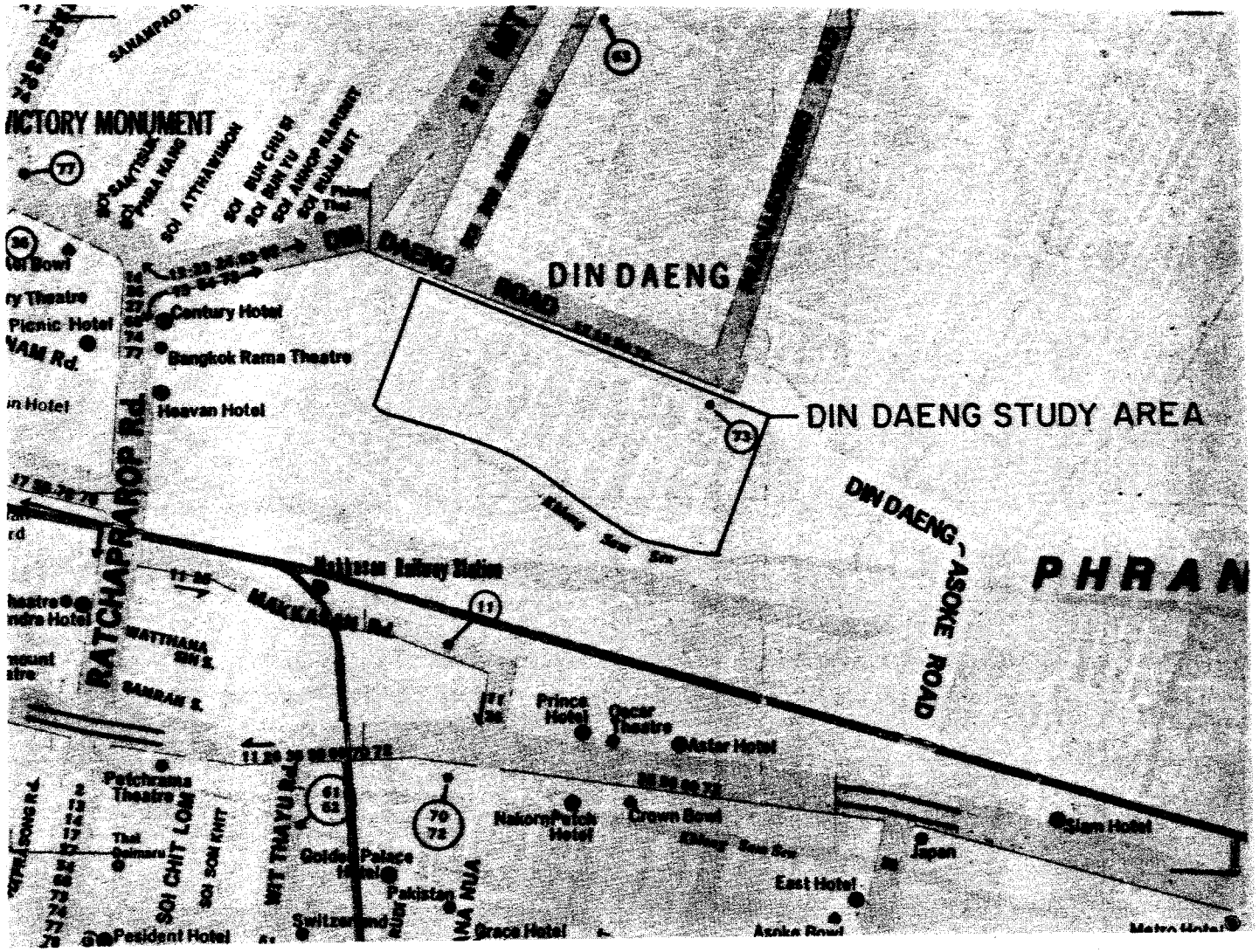

Figure 1. Dengue virus ecology and epidemiology study area, Bangkok, Thailand.

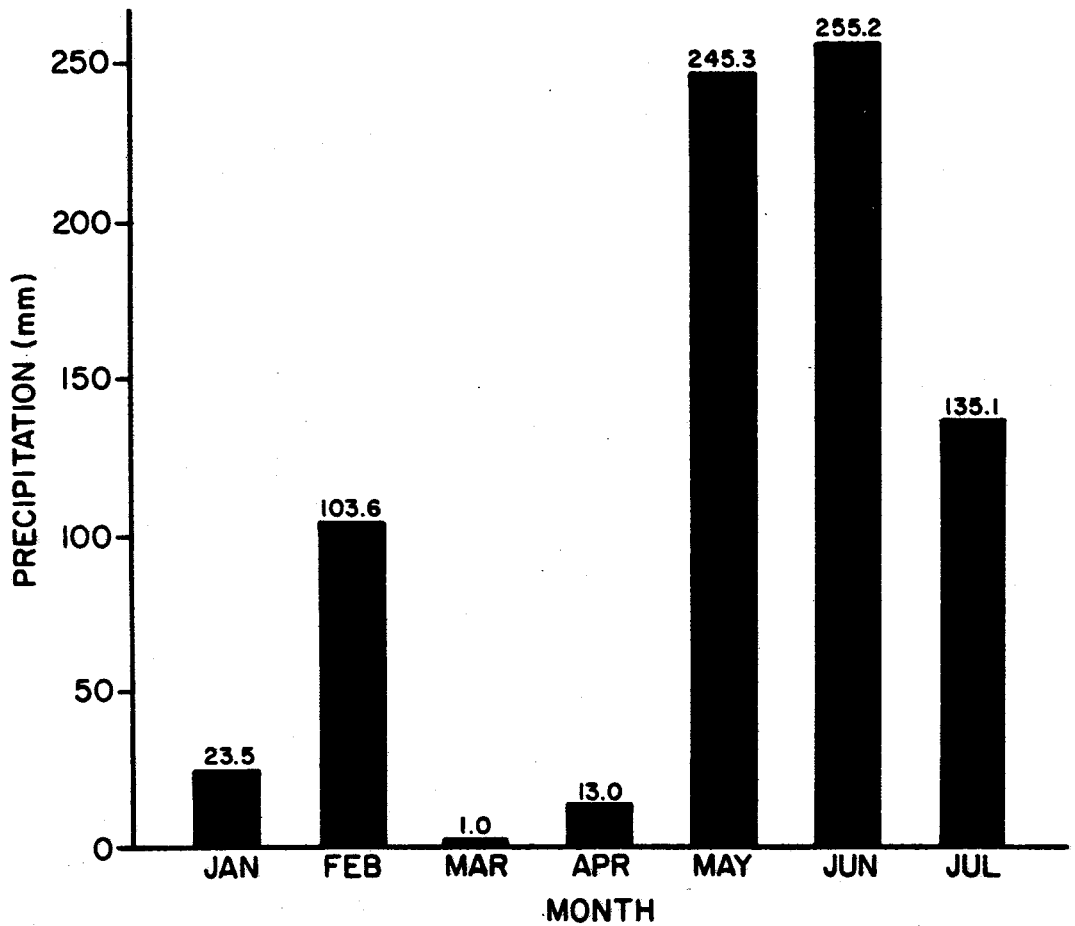

FIGURE 2. AMOUNT OF PRECIPITATION FOR JANUARY THURGH JULY, 1978, BANGKOK, THAILAND.

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